論文 / 著書情報
Article / Book Information

題目(和文)	Cenococcum geophilumおよび近縁種の菌核の分布特性と土壌腐植構成成分としての役割
Title(English)	Distributional properties on sclerotia of Cenococcum geophilum and related species, and their role as a soil organic component
著者(和文)	坂上伸生
Author(English)	Nobuo Sakagami
出典(和文)	学位:博士（理学）, 学位授与機関:東京工業大学, 報告番号:甲第7137号, 授与年月日:2008年3月26日, 学位の種別:課程博士, 審査員:渡邊 眞紀子
Citation(English)	Degree:Doctor of Science, Conferring organization: Tokyo Institute of Technology, Report number:甲第7137号, Conferred date:2008/3/26, Degree Type:Course doctor, Examiner:
学位種別(和文)	博士論文
Type(English)	Doctoral Thesis

Distributional properties on sclerotia of *Cenococcum geophilum* and related species, and their role as a soil organic component

by

Nobuo SAKAGAMI

Supervisor: Professor Makiko WATANABE

Department of Environmental Science and Technology

Interdisciplinary Graduate School of Science and Engineering

Tokyo Institute of Technology

March 2008

Contents

Chapter 3. Interaction and contribution to soil aluminum and iron

Chapter 4. Spatial distribution and regulating factor of formation of sclerotia in low pH forest soils

Chapter 1

Introduction – sclerotia in soils

1.1 Sclerotia

Sclerotia are fungal resting structures, or persistent propagules which can germinate to produce mycelia, asexual spores of sporocarps such as apothecia in which sexual spores are borne (Coley-Smith and Cooke, 1971). Sclerotia are considered to be resistant against microbial attack during the period (generally one month to several years) of inactivity as well as to desiccation (Cochrane, 1958; Gray and Williams, 1971; Fox, 1986). According to Townsend and Willetts (1953), many diseases of economic plants are caused by those fungi which normally produce sclerotia at some time in their life history. These sclerotia were recognized with great biological and phytopathological importance since they serve themselves as vegetative reproductive bodies, or reproductive structures developed from themselves. Moreover, their structure enables them to survive periods of adverse conditions which are too severe for the ordinary vegetative mycelium.

Sclerotia have attracted much research interests on botany, mycology, cytology, histochemistry, and phytopathology. Many researches from these interests were reported on species such as *Rhizoctonia solani* (Naiki and Ui, 1975, 1977), *Sclerotinia sclerotiorum* (Maxwell *et al*., 1970; Saito, 1977), *Sclerotinia minor* (Bullock *et al*., 1980a, 1980b, 1983), *Sclerotinia fructigena* (Calonge, 1986), *Sclerotium rolfsii* (Chet *et al*., 1967, 1969), *Pisolithus tinctorius* (Grenville *et al*., 1985a), *Hebeloma sacchariolens* (Fox, 1986), *Typhula incarnata* and

T. ishikariensis (Matsumoto and Tajimi, 1988, 1990). Some mycorrhizal fungi (e.g. *Paxillus involutus*, *Cenococcum geophilum*) are also known to form sclerotia (Trappe, 1964, 1969; Grenville *et al*., 1985b; Fox, 1986, Moore *et al*., 1991; Massicotte *et al*., 1992). Fig. 1.1 and Fig. 1.2 are scanning electron micrographs of sclerotia of *P. involutus* (Grenville *et al*., 1985b) and *C. geophilum* (Massicotte *et al*., 1992).

Early stage in development of sclerotial initiation, ordinary growth of mycelium (branching, fusion of branches, coalescence and septation) occurs intensively (Townsend and Willetts, 1953). Sclerotia of *S. rolfsii* are known to have involuted structure such as rind, cortex, medulla, and an intermediate layer (Chet *et al*., 1969). On the contrary, simple sclerotia formed by *R. solani* have uniformed structure constructed with almost ordinary cells of mycelium.

These studies place interests for mycological and physiological processes. Thus, relatively few studies have been reported on geographical distribution of sclerotia. Matsumoto and Tajimi (1988, 1990) reported life-history strategy determined by sclerotium production and continuous variation associated with habitat differences within isolates of *T. incarnata* and *T. ishikariensis*. Naiki and Ui (1977) reported the population of sclerotia of *R. solani* in soil, which causes “crown rot” disease of sugar beet. Most of sclerotia distributed in rhizosphere and surface soil, and the highest number of sclerotia per soil observed in the closest to the roots of infected plants (Fig. 1.3).

Sclerotia are also known to contain “reserve substances” for germination, such as lipids in *C. geophilum* (Massicotte *et al*., 1991), *P. tinctorius* (Grenville *et al*., 1985a), *P. involutus* (Grenville *et al*., 1985b; Moore *et al*., 1991), protein in *P. involutus* (Grenville *et al*., 1985b; Moore *et al*., 1991), and carbohydrates in both *P. tinctorius* (Grenville *et al*., 1985a) and *P. involutus* (Grenville *et al*., 1985b; Fox, 1986; Moore *et al*., 1991). Moore *et al*. (1991) reported the chemical composition of reserve substance and cytoplasm of the cells of *P. involutus*. Protein bodies (electron opaque granules) were characterized by phosphorus (P) and accompanying cations (Mg, S, Ca, Ni), and cytoplasm were characterized by lower levels of P,

Ca and Mg (Fig. 1.4).

1.2 *Cenococcum geophilum*

C. geophilum is one of the most frequently encountered ectomycorrhizal (ectoendomycorrhizal) fungi in nature (LoBuglio, 1999). It has a worldwide distribution in temperate and arctic-alpine climatic zones, and forms ectotrophic and ectendotrophic associations with unusually large numbers of tree, shrub and herbaceous genera (Trappe, 1964). Fig. 1.5 shows a "jet-black" sclerotia and hyphae associating with *Quercus crispula* root. According to LoBuglio (1999), *C. geophilum* was originally described from its black sclerotia by J. Sowerby in 1800 under the name *Lycoperdon graniforme*. LoBuglio *et al*. (1991) reported that *C. geophilum* exists as a sterile dematiaceous mycelium and thus lacks sexual or asexual spores, which are important taxonomic criteria in fungal classification, and thus it might be a very heterogeneous species or a fungal complex representative of a broader taxonomic rank.

In addition to its broad habitat range, *C. geophilum* is known to have a pioneering ability, which reported that it formed ectomycorrhiza with pioneering seedlings of *Pinus albicaulis* and *Larix lyalli* on recently exposed moraines in the Oregon Cascade mountains (Trappe, 1988). Other fungi may also invade in certain situations such as sand dunes, glacial moraines, volcanic ash, cinders, pumice, and clear-cut areas, and become more successful than *C. geophilum* as environmental conditions gradually improve (LoBuglio, 1999). Shaw and Sidle (1982) reported that the ability of live sclerotia to survive for several years can provide sufficient inoculum to colonize host species effectively. Such wide distribution can be understood from the capability of *C. geophilum* against adverse and biotoxic condition. For example, *C. geophilum* still increased in soils under exposure of simulated rain at pH 2.5 (Meier *et al.*, 1989).

1.3 Sclerotia of *Cenococcum geophilum*

Trappe (1969) noted sclerotia of *C. geophilum*, which tend to be particularly abundant near *Cenococcum* mycorrhiza, as 0.05~4 mm or more in diameter, and viable, mature ones are jet-black, hard, smooth, mostly spherical, and often with emergent hyphae (Fig. 1.6). He also noted live sclerotia are denser than water, and have a high content of ethanol-soluble oil (supposedly reserve substances), while dead sclerotia – which can persist indefinitely in soil – look much like live ones but float in water and lack the ethanol-soluble oil. Sclerotia are easily found from forest soils (Fig. 1.7). The morphological features of sclerotia can be observed by slicing into half pieces using a sterilized knife. As shown in Fig. 1.8, they have honeycomb structure in their transverse wall constructed by coalescenced empty cells. Histopathologicaly involuted structure can not be recognized, but a hollow structure can be frequently recognized inside sclerotium.

Sclerotia of *C. geophilum* can significantly contribute to the fungal biomass of forest situations, and thus represent an important source of assimilated carbon (C) from host species (LoBuglio, 1999). Vogt *et al*. (1981) estimated the biomass of sclerotia in a 23- and 180-year-old *Abies amabilis* stand to be 2300 kg ha^{-1} $year^{-1}$ and 3000 kg ha^{-1} $year^{-1}$, respectively. Physical persistence of vesicular-arbuscular mycorrhizal spores (grains of fungal products like sclerotia) was demonstrated in research on paleosols. Holmqvist and Schlyter (2000) examined the chronosequense of closely superimposed paleosols from an arctic/alpine meadow soil in northern Sweden with radiocarbon (^{14}C) dating of asexual spores of vesicular-arbuscular mycorrhizae, and determined the long-term (i.e., over a millennium time span) loss rate of organic C.

From the viewpoint of soil genesis and soil organic chemistry, sclerotia of *C.*

geophilum were assumed as one of the source of "Pg", the green fraction of humic acid by Kumada and Hurst (1967). They examined sclerotia of *C. geophilum* (*C. graniforme*) in British and Swedish Podsols and showed that the distribution of sclerotia was larger in A or B horizon.

Watanabe *et al*. (2001) examined the chemical properties of sclerotial grains collected from a buried humic horizon of volcanic ash soils in Mount Myoko, central Japan, where Kumada (1987b) noted abundant existence of large sized (over 7 mm in diameter) sclerotia, using a scanning electron microscope (SEM) and energy dispersion X-ray fluorescence micro-analyzer (EDX), an electron probe micro-analyzer of wave dispersion type (EPMA) and ^{27}Al magic-angle spinning (MAS) nuclear magnetic resonance (NMR) analyses. They noticed that C was the major element in sclerotia associated with a relatively large concentration of octahedral Al, which suggested an Al-humus complex. By further investigation on the distributions of sclerotia in profiles of Japanese Andosols and German Podsols, Braunfahlerde and Brown Podsols, Watanabe *et al.* (2002; 2004b) concluded that formation of sclerotia was regulated by the content of exchangeable Al and the status of active Al in the soil, regardless of soil type. From the studies on micro-morphological features and chemical compositions inside the grains using transmission electron microscopy (TEM), SEM and EDX techniques (Watanabe *et al*., 2004a), the Al-oxyhydroxide polymorphs found in grains were assumed to be formed by Al dissolution- precipitation inside the grain, which can be considered to be induced by biochemical processes of host fungi or bacterium in sclerotia. In addition, the existence of heavy metals such as Ti, Ni, and Pb was observed in the sclerotia. The ^{14}C ages of sclerotia from surface A horizons and buried A horizons of *Fagus crenata* forest soil were reported as ca. 100~200 yr. B.P. and ca. 300~1,200 yr. B.P., respectively (Watanabe *et al*., 2007). Moreover, the ^{14}C ages of sclerotia were older than those of humic acids and soil, and thus they revealed its persistence for a long term as a structural organic component in soils. These studies suggested close associations of sclerotia to formation of soil humic substances and exhibited certain importance as soil organic component.

1.4 Objective, structure of this study

As reported by LoBuglio *et al*. (1991), *C. geophilum* is considered as a highly heterogeneous species or a fungal complex representative of a broader taxonomic rank. But since sclerotia are frequently abundant in soils containing *Cenococcum* mycorrhizae, studies on sclerotia of *C. geophilum*, supposedly including related species, will serve important information for soil-biology and soil-science.

The objective of this study is to clarify distributional properties of sclerotia and its role as a soil organic component. The structure of this study is shown in Fig. 1.9.

Chapter 1 is the general introductive section for sclerotia, *C. geophilum*, sclerotia of *C. geophilum* to lead distinction of the objective grains in this study. In chapter 2, characteristics of sclerotia are examined with their physical properties, functional C groups and elemental composition. In chapter 3, interactions between sclerotia and soils are discussed with characteristics of Al and Fe in sclerotia and soils. From these information, definition of sclerotia as soil organic component is discussed.

In chapter 4, spatial distribution of sclerotia is performed in low pH soil in *Picea abies* forest. In chapter 5, seasonal change of distribution of sclerotia, and distribution along an altitudinal gradient in temperate and arctic-alpine climatic zones are demonstrated in central and northern Japan. Regulating factors of formation and significance of sclerotia as a soil organic component are discussed to lead understanding on strategies of *C. geophilum* to form sclerotia.

Chapter 6 gives brief conclusions on existing implications and roles of sclerotia as soil organic component, and proposes a dynamic model of formation of sclerotia of *C. geophilum* and related species.

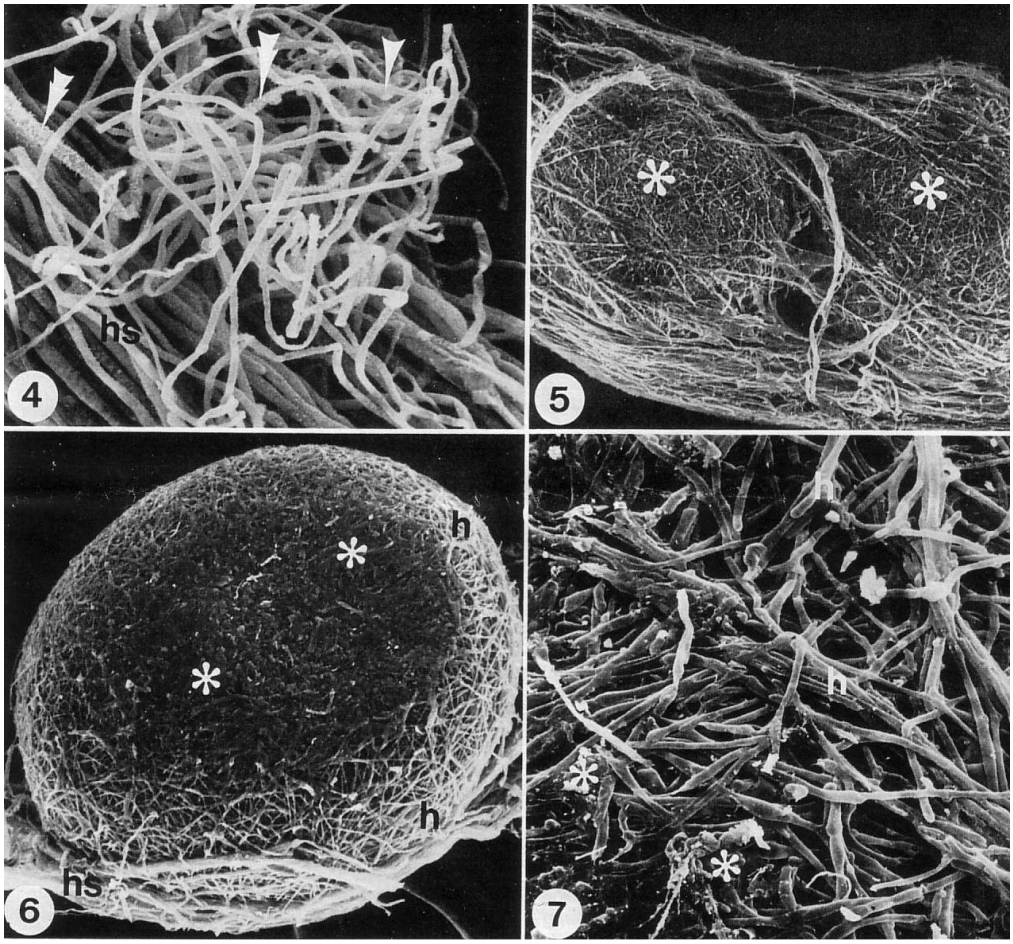

Fig. 1.1. Scanning electron micrographs of sclerotia of *P. involutus* (Grenville *et al.*, 1985b)

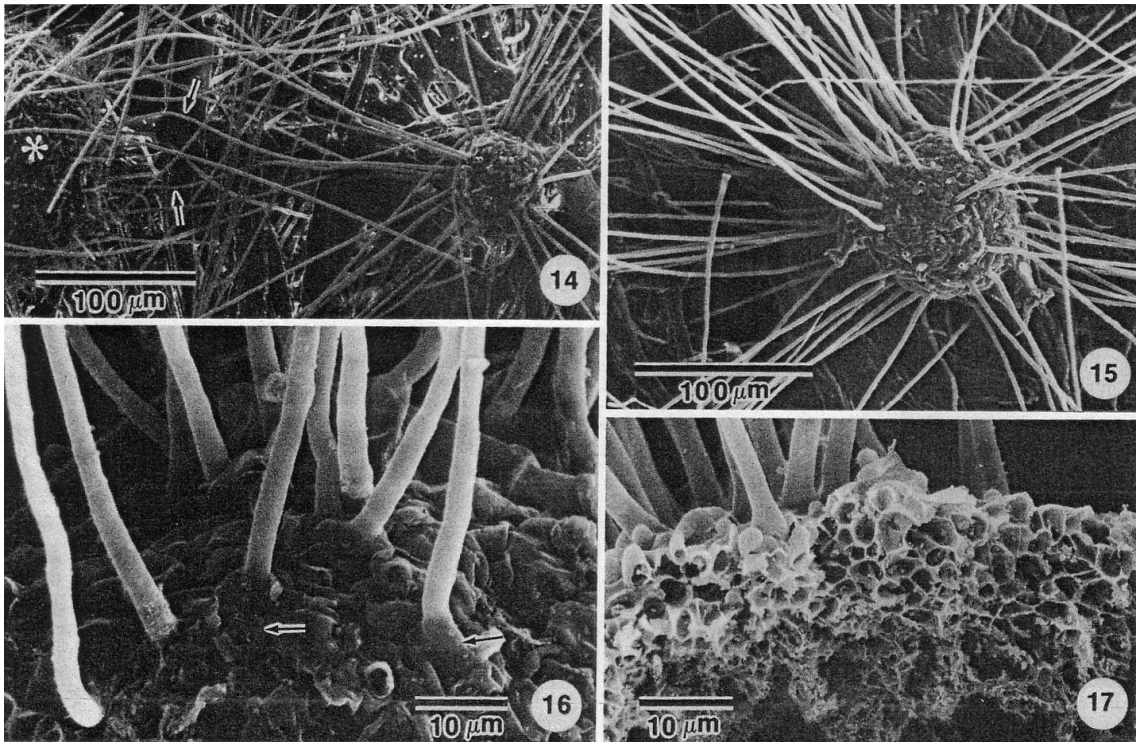

Fig. 1.2. Scanning electron micrographs of sclerotia of *C. geophilum* (Massicotte *et al*., 1992)

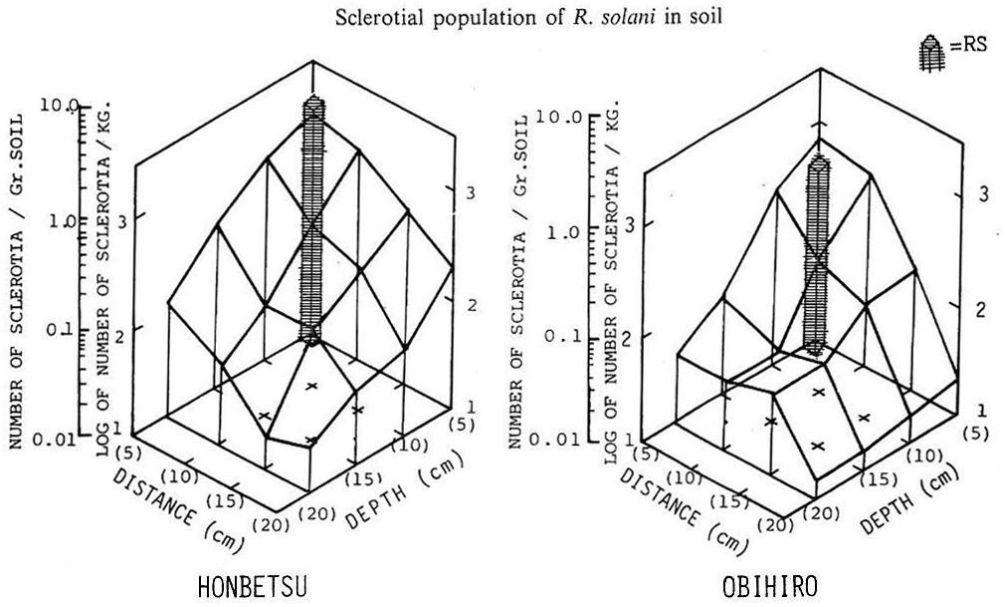

Fig. 1.3. Distribution of sclerotia of *R. solani* (Naiki and Ui, 1977).

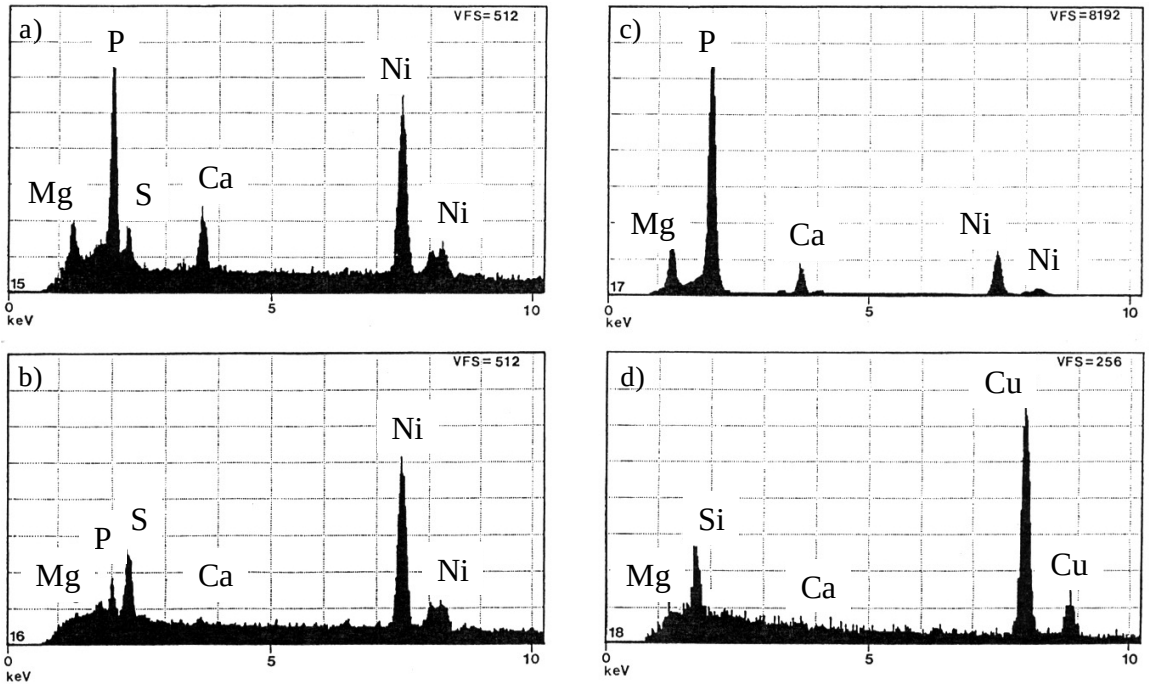

Fig. 1.4. Spectra of EDS analyses on a), b) protein bodies and c), d) cytoplasm of cells of *P. involutus* (Moore *et al*., 1991). VFS indicates vertical full scale.

Fig. 1.5. “Jet-black” sclerotia and hyphae of *C. geophilum* associating with *Quercus crispula* root.

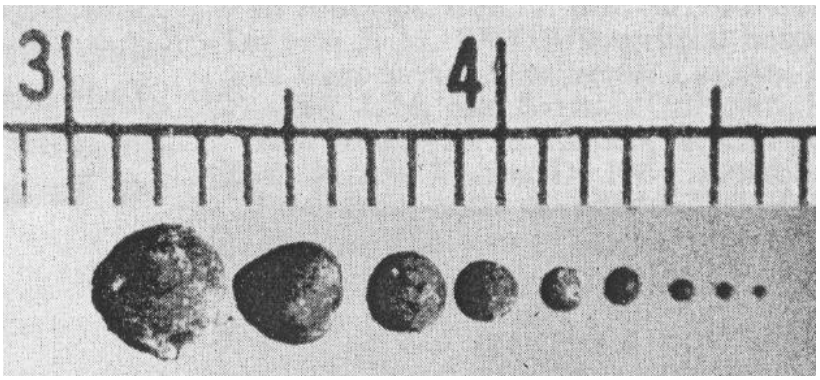

Fig. 1.6. Sclerotia of *C. geophilum* noted by Trappe (1969).

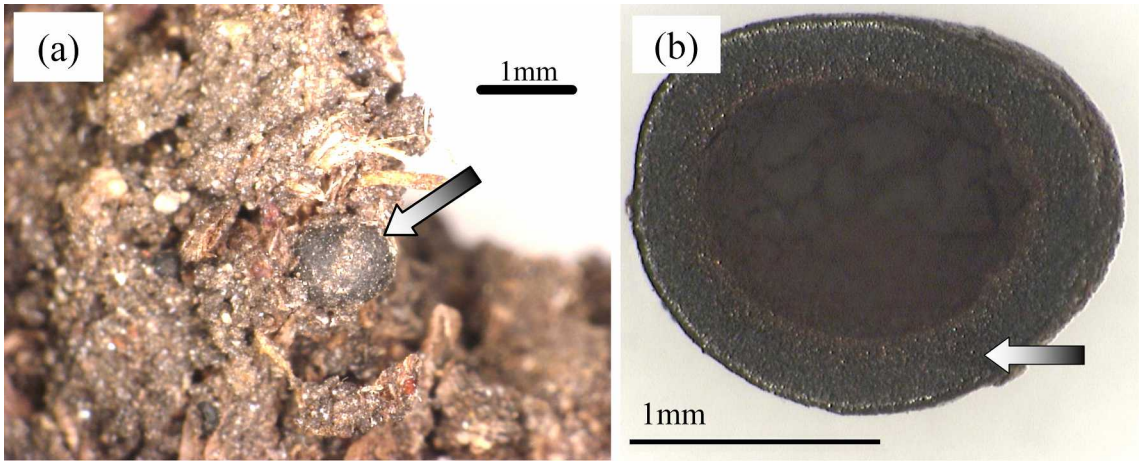

Fig. 1.7. (a); Sclerotium of *C. geophilum* (arrowed), found in forest soils. (b) Internal structure of a sclerotium from Mt. Myoko. Arrow denotes the transverse wall.

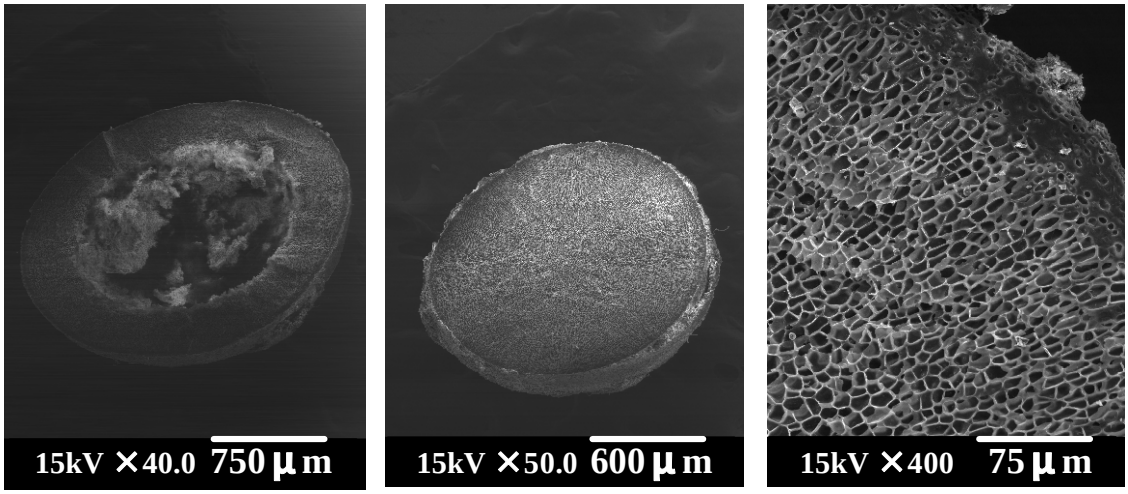

Fig. 1.8. The morphological feature of sclerotia of *C. geophilum* observed by scanning electron microscopy. The grains were obtained from Ramberg Ah horizon [a) and c)] and Brandhai Ah horizon [b)], in Harz Mts, Germany. a) and b), internal features; c), surface structure of the transverse wall (from Watanabe *et al*., 2004b).

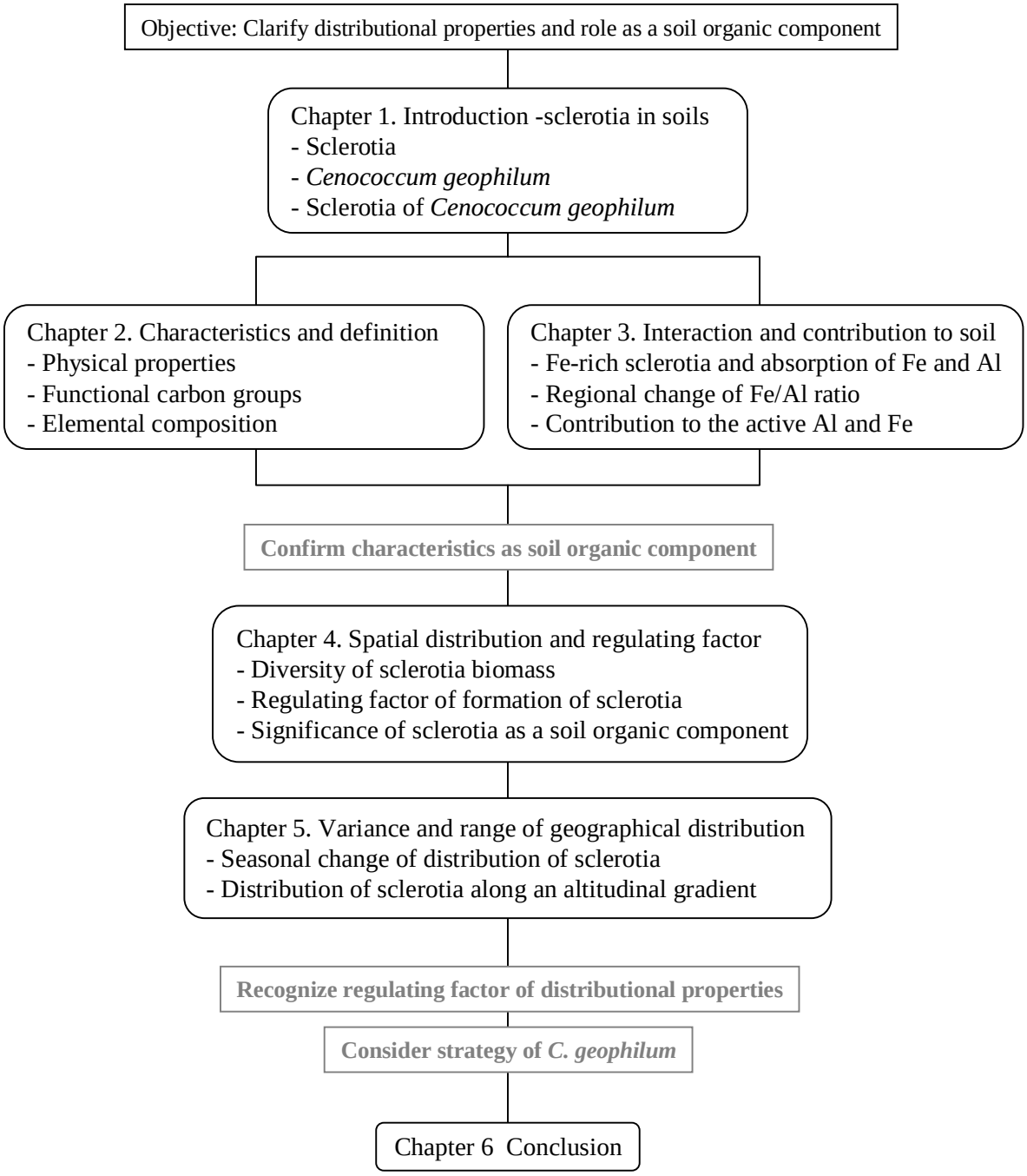

Fig. 1.9. Structure of this study.

Chapter 2

Characteristics and definition of sclerotia as a soil organic component

2.1 Introduction

Watanabe *et al.* (2001, 2002) reported high concentrations of Al in sclerotial grains of *C. geophilum* and the possible occurrence of Al-humus complexes inside the grains. A positive relationship was obtained between the mean weight of the sclerotia and the content of exchangeable Al (Al_{Ex}), which is potentially phyto-toxic in forest soils with andic properties. Al_{Ex} contents in soils were determined as a regulating factor of formation of sclerotia in study on Harz Mts. area (Watanabe *et al*. 2004b).

Kumada (1987b) noted abundant existence of sclerotia in Myoko soil. The maximum content of sclerotia in Myoko soil is 3.4 g kg^{-1} (Watanabe *et al*., 2001; 2002; Sakagami *et al*., 2004), which suggests that the contribution of sclerotia to forest soils cannot be ignored as one of the soil organic components. In this chapter, physical and chemical analyses were examined on sclerotia from Myoko and other forest soils to clarify the characteristics and to define sclerotia as soil organic component.

2.2 Materials and methods

Fig. 2.1 shows the study areas in this chapter. Sclerotia used in this study were mainly taken from the 3A horizon (27~37 cm depth; Fig. 2.2) of the Mt. Myoko soil (Fulvic Andosol, WRB/FAO-Unesco), Niigata pref., Japan (36°54'00"N, 138°08'25"E; altitude: 1320 m) as described in Watanabe *et al.* (2001). Mean temperature and annual precipitation in the area are 5.8 ºC and 2280 mm, respectively. Vegetation of the site was characterized by the presence of *Fagus crenata* and *Sasa kurilensis*. Sclerotia were also collected from Mt. Rishiri (Hokkaido pref.), Lake Tazawa plateau (Akita pref.), Mt. Higashi-adzuma (Fukushima pref.) and Mt. Ontake (Gifu pref.) soils to clarify the variance of composition of major elements (C, H, N, O and ashes). In Lake Tazawa plateau, sclerotia were collected from three different points: two beech forest (Tazawa F1 and F2) and one cedar forest (Tazawa C). In Mt. Rishiri, sclerotia were collected from two different elevations: 500m (Rishiri 500) and 1000m (Rishiri 1000). In Mt. Ontake, soil profile was investigated and sclerotia were collected from each horizon (A1, A2, A/E, Bh and Bw layers)

Sclerotia were directly separated from the collected soils by hand picking using tweezers in the laboratory and were air-dried at 25 ºC for 1 week to examine physical properties, elemental composition and functional carbon (C) groups of grains. Sclerotia for elemental composition and functional C groups analyses, were placed into a 7×10 cm plastic bag with 50 ml distilled water in order to clean the surfaces ultrasonically (US-1 generator, Iuchi (AS ONE) Corp., Osaka; 70 W, 38 kHz, 5 minutes). The cleaned grains were air-dried at 25 ºC for 1 week, and then were ground to a fine powder (<5 μm) in a clean agate mortar. Powder samples of sclerotia from Myoko soil were differentiated into three groups. Myoko group 1: grains from the June 2002 soil, and Myoko group 2 and 3: grains from the June 2003 soil separated by grain size, 1~2 and 2~5 mm, respectively.

The weight and diameter of grains were measured using an electronic balance and a digital high density video microscope (VH-7000, Keyence, Osaka), respectively. Forty five

spherical grains and sixty one grains of other feature were measured and bulk densities were calculated by their weight and volume. True density of sclerotia was measured by applying "Test method for density of soil particles" (JIS A 1202: 1999; Japanese Geotechnical Society, 2000), using another 1.7 g of grains. Sclerotial grains with 50 ml pycnometer were used for the test and it was repeated three times. True density of sclerotia was calculated as follows:

$$\rho_s = \frac{m_s}{(m_s + m_a - m_b)} \times \rho_w (T) \qquad (1.1$$

where ρ_s is true density of sclerotia, m_s is weight of sclerotia samples, m_a is weight of pycnometer filled with pure water, m_b is weight of pycnometer filled with samples and pure water, and ρ_w (T) is density of water at T °C.

Solid-state cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR spectra were recorded with a FT-NMR system (Alpha 300, JEOL Ltd., Tokyo). Approximately 100 mg of powdered sample from Myoko group 1 was transferred into KEL-F spinning tubes (6 mm diameter, JEOL Ltd., Tokyo). The solid-state CPMAS ^{13}C NMR signals were recorded at 75.45 MHz with 6 kHz of magic angle spinning, 1 ms contact time, and a 3 s pulse interval. A broadening factor of 100 Hz was employed in the Fourier Transformation procedure. Standard chemical shift (0 ppm, tetramethylsilane) adjustment was performed using adamantane (29.50 ppm).

The contents of C, H, and N in sclerotia were determined by a dry combustion method (CHN analyzer, type MT-6, Yanaco Corp., Kyoto). About 2 mg of powdered sample was used for the measurements. The oxygen content was evaluated as the difference between the amount of sample (2 mg) and the total weight of the ash plus the sum of C, H and N. The water content was obtained by subtracting the weight of the 105 °C oven-dried sample from that of the air-dried sample (about 2 mg). All measurements were repeated twice for each group. The atomic ratios of O/C and H/C were calibrated to a water- and ash-free basis.

Quantitative analysis of content of trace element in sclerotia was carried out by using a Particle Induced X-ray Emission Spectrometer (PIXE) at Tokyo Institute of Technology. Between 20~25 mg of powdered sample, taken twice from each Myoko group, were placed in a beaker with 0.3 ml of ultra pure nitric acid (HNO_3) for about 5 minutes in a microwave oven at 120 °C. Then 0.1 ml of 30% (v/v) hydrogen peroxide (H_2O_2) solution was added to the residue and was heated at < 100 °C for 2 minutes until almost evaporated, whereupon 0.6 ml of 0.1 M HNO_3 was added. Finally, the solution was again evaporated almost to dryness at 130~140 °C. The residue was dissolved in 1 ml of 0.1 M HNO_3 to produce a transparent clear solution. After digestion, the samples were treated to prepare targets. 20 μl of 1000 mg l^{-1} Mo standard (internal standard) was added to the 1 ml of the sample solution, and then a 20 μl aliquot of this mixture was dropped directly onto Nuclepore Track-Etch Membrane (Whatman, New Jersey, USA), air-dried, and irradiated by the 2.5 MeV proton beam from a single-end type Van de Graaff accelerator (Tsuji *et al.*, 2000). The proton beam was collimated to about 5 mm diameter at the collimator exit. Emitted X-rays were analyzed using an ORTEC (Tennessee, USA) Si (Li) detector (active volume: 0.45 cm^3, effective diameter: 10mm, thickness: 5.67 mm, distance from window: 7 mm, full width half maximum (FWHM) of Fe K_α radiation: 180 eV) with a 1-mm polyethylene absorber and a CANBERRA (Meriden, USA) Si (Li) detector (effective diameter: 4 mm; thickness: 3 mm, distance from window: 5 mm, FWHM of Fe K_α radiation: 160 eV) without an absorber. The beryllium window was 25 μm thick for both detectors. The first detector was used for measurement of heavy elements (Z > 25) and the second for light elements (Z < 25). All the chemicals were supplied by Wako Pure Chemical Co. Ltd. (Osaka), and used without further purification.

The PIXE analysis proceeds in three major steps; digestions of sample, target preparation, and measurement of X-ray intensity. As it is instructive to evaluate the magnitudes of the variance in experimental results induced by each step, the dispersion or relative standard deviation of individual samples around the mean was measured as the variance (s^2) according to

Montgomery (1991). The variance, or relative standard deviation $s_{r\sum}$ was calculated as follows:

$$s^2_{r\sum} = s^2_{rr} + s^2_{rt} + s^2_{rs} \quad (1.2$$

where s^2_{rr} is the repeatability of analytical results for the solutions and depends on instrumentation stability, including the stability of the positioning of a sample in the spectrometer; s^2_{rt} is the stability of the target preparation, including the effect of introduction of an internal standard; and s^2_{rs} is the quality of the preparation of solutions from the sclerotium sample. Each solution was prepared twice to isolate these error components. For each solution, two aliquots were taken, an internal standard was introduced, and finally, a target was prepared from each aliquot. Each target was measured twice with the sample positioned independently in the spectrometer.

2.3 Physical properties of sclerotia

Fig. 2.3 shows the relationships between diameter and volume, and weight of sclerotia from Myoko soil. From this result, bulk density of sclerotia were calculated by volume and weight and ranged from 0.29~1.2 g cm^{-3} in case of spherical grains. This variance was considered to represent grades of hollow structure. True density of sclerotia was obtained as 1.512 ± 0.026 g cm^{-3}.

According to NAOJ (National Astronomical Observatory of Japan, 2003), bulk and true density of charcoals are 0.3~0.6 and 1.4~1.9, respectively. In addition, true density of sugar, hemp and cotton are 1.59, 1.50~1.52, 1.50~1.55, respectively. It is instructive to compare these densities of sclerotia and these organics. Characteristic of density of sclerotia were corresponded to such “natural” organics, which are normally exist as soil organic component.

2.4 Functional carbon groups of sclerotia

Fig. 2.4 shows the solid-state CPMAS ^{13}C NMR spectrum for Myoko sclerotia. The spectrum for humic and fulvic acids extracted from an allophanic Andosol are given for comparison. The assignments of specific peaks (A-F) in Fig. 2.4 are: (A) carboxylic and carbonyl C; (B) aromatic C; (C, D) *O*-alkyl C; (E) methoxyl C; (F) alkyl C. The spectrum shows a large proportion of *O*-alkyl C in sclerotia. This points to the dominance of sugar chains, and reveals a completely different spectrum from humic and fulvic acids of an allophonic Andosol, which consists of relatively large proportions of carbonyl and carboxyl C. The aromatic carbons of sclerotia also showed a distorted pattern from those of the humic acid of the Andosol (Hiradate *et al*., 2004). Methyl C in sclerotia (peak F) was considered to originate from deoxy-sugars such as rhamnose.

Sclerotia are considered to be one of the precursors of the humic acid "Pg" fraction (Kumada and Hurst, 1967). The analyses of purified humic acid "Pg" (Watanabe *et al*., 1996) extracted from soils in Mt Myoko, showed similar chemical compositions to the sclerotia. As Watanabe *et al*., (1996) reported that their fraction G1 contained larger amounts of alkyl chains while their fraction G2 contained larger amounts of polysaccharides, the chemical features of C species in sclerotia were more likely to be those of combined G1 and G2 fractions.

2.5 Elemental composition of sclerotia

2.5.1 CHNO composition of Myoko sclerotia

Contents of C, H and N ranged from 46.2~48.4, 3.12~3.52, and 0.72~0.82 %, respectively. The ash and water contents in the samples ranged from 10.5~12.8, 4.85~8.25 %, respectively. As there was no significant difference in CHN contents of the Myoko sclerotia by grain size, the mean contents of major elements, ash, and water in these grains were obtained for the 6 powder samples as shown in table 2.1. Mean contents of C, H, N, O, and ash of sclerotia were thus estimated as 47.6, 3.32, 0.78, 30.2, and 6.57%, respectively. The water content in the samples was 11.6%. The atomic ratios of O/C and H/C calculated to water- and ash-free basis were 0.48 and 0.83, respectively.

2.5.2 Trace element composition by PIXE analysis

Fig. 2.5a and b show the X-ray spectra of sclerotia in low (Z < 25) and high (Z > 25) energy regions, respectively. Fig. 2.5a shows the major components of sclerotia. From Fig. 2.5b, a clear peak for Pb along with other transition and alkaline earth metals is confirmed. Since the energy of Pb L_{α} and As K_{α} X-rays are 10.54 and 10.53 keV, respectively in these spectra, As and Pb are difficult to separate. In order to interpret the Pb peak, the peak of As K_{β} were checked, which was not clear in the sample, while clear peaks were confirmed for both Pb L_{α} and Pb L_{α} energy lines.

The element concentrations in sclerotia and the results of the variance analysis are summarized in table 2.2 and table 2.3, respectively. The concentrations of Al, Fe, Na, and Ca were 1.4, 0.57, 0.16, and 0.14%, respectively, and those of the trace elements Si, P, S, K, Ca, Ti, Cr, Mn, Cu, Zn, Br and Pb were between 10 and 100 μg g^{-1}. The maximum and minimum contents of the elements detected are shown in the table 2.3. The results of variance analysis showed that the main contribution in the dispersion is related to reproducibility of analytical results for solutions and depends on the measurement instrumentation. The largest dispersion (51%) is for Cl and the least for Al (4.6%). The concentrations of Cl, Ni, and Sr showed

relatively large variances caused mainly by solution preparation. Consequently, it was considered that Cl, Ni, and Sr are not present in sufficient quantity to determine their concentrations reliably in this study. As there is no standard sample for conducting element analysis of sclerotia, the variance analysis seems to be helpful in planning future experiments to achieve low variance for trace elements in solution.

Fig 2.6 shows the compiled elemental composition of CHNO and trace element of Myoko sclerotia including water content. Elemental composition of sclerotia was characterized by approximately half of C, and relatively high contents of ashes (i.e. Al and Fe). Low content of N, P, and S suggests that sclerotia do not exist as mycelia, but mostly as an abiotic organic component.

2.5.3 Variance of CHNO composition

Fig. 2.7 shows the results of CHNO compositions of sclerotia from surface soils. C content ranged 43.8~53.6% and ash content had negative correlation with C content. Fig. 2.8 shows results of CHNO compositions of sclerotia from each horizon of Ontake profile. Sclerotia from lower layer tended to have lower C content. These variances may be able to be explained by their soil-environmental conditions.

Since the honeycomb structure of sclerotia is constructed by coalescenced empty cells, they supposedly originated from fungal cell wall, chitin. Fig. 2.9 shows the atomic ratio of O/C and H/C of sclerotia, chitin, humic and fulvic acid. The data of humic and fulvic acids were referred from Kumada (1987a). Sclerotial O/C and H/C distributed between humic acid and chitin. This fact suggests that the sclerotia are transitive component from fungal metabolites to humic acids. This hypothesis will harmonize with formation of Al-humus complex in sclerotia reported by Watanabe *et al*. (2001). Stevenson (1994) suggested “Polyphenol theory” of humus formation (Fig. 2.10). Adjusting to this theory, sclerotia can be recognized as en route toward

humic acids from “cellulose and other non-lignin substrates”. Bacterial community analysis was performed with sclerotia sampled from an Andosol profile in Mt. Myoko and revealed the predominance of Alphaproteobacteria, chiefly the *Sphingomonas* group (Ohta *et al*., 2003). This fact will support “utilization by micro-organisms” in sclerotia.

2.6 Conclusion

The mean concentrations of major elements in sclerotia from Mt. Myoko were C (47.6 %), O (30.2 %), H (3.3 %), Al (1.4 %), N (0.78 %) and the atomic ratios of O/C and H/C were 0.48 and 0.83, respectively. Carbon in sclerotia was in the form of large amounts of *O*-alkyl C and also associated with aromatic C and methyl C, which showed strongly and characteristically biological origin, and completely different spectral features to humic and fulvic acids from an allophonic Andosol. On the contrary, low content of N, P, and S suggested that sclerotia do not exist as mycelia, but as an abiotic organic component.

The variation of CHNO, and ash content of sclerotia were likely to reveal their soil-environmental conditions. Sclerotia were suggested as transitive component from fungal metabolites to humic acids, en route toward humic acids from “cellulose and other non-lignin substrates”. In next chapter, chemical properties of sclerotia in relation to the status of metals, especially of Fe and Al, are examined to understand their contribution to soil-environmental condition.

Table 2.1. Contents of major elements (C, H, N, O), ash and, water in sclerotia, and atomic ratios of [O]/[C] and [H]/[C].

	Major elements / wt %				Ash / wt %
	C	H	N	O	
Sclerotia	47.6	3.32	0.79	30.2	6.57
(n=6)	Water content / wt %		Atomic ratio (Water- and ash-free)		
	H_2O		[O]/[C]		[H]/[C]
	11.6		0.48		0.83

Table 2.2. Element concentrations in sclerotia by PIXE analysis.

Element	Sclerotiua (n=6)
	μg g^{-1}
Na	1637
Al	14376
Si	807
P	248
S	845
Cl	56.1[a]
K	386
Ca	1439
Ti	100
Cr	32
Mn	39
Fe	5688
Ni	29[a]
Cu	55
Zn	87
Br	19
Pb	38
Sr	48[a]
Total	25929

[a] Concentration values of Cl, Ni, and Sr are not significant enough to determine reliably based on the results from Table 2.3.

Table 2.3. Result of the dispersion analysis with PIXE

Element	Concentration		Variance			
	Min	Max	s^2_{rr}	s^2_{rt}	s^2_{rs}	$s^2_{r\Sigma}$
	$\mu g\ g^{-1}$		%			
Na	900	2200	7.4	8.8	0	11
Al	9700	18800	4.6	0	0	4.6
Si	345	1380	9	24	0	26
P	189	337	15	0	0	15
S	488	1130	9.3	0	8.3	12
Cl	5.8	19.2	29	0	41	51
K	308	635	11	0	0	11
Ca	956	1846	3.2	7.2	0	7.9
Ti	45	158	13	0	0	13
Cr	15	73	13	25	0	28
Mn	8	88	13	27	0	30
Fe	4760	7220	5.6	0	0	5.6
Ni	3	6	28	0	30	41
Cu	30	66	9.3	0	11	14
Zn	50	180	5.3	22	0	23
Br	10	31	27	0	0	27
Pb	30	50	10	12	0	16
Sr	30	75	11	0	28	30

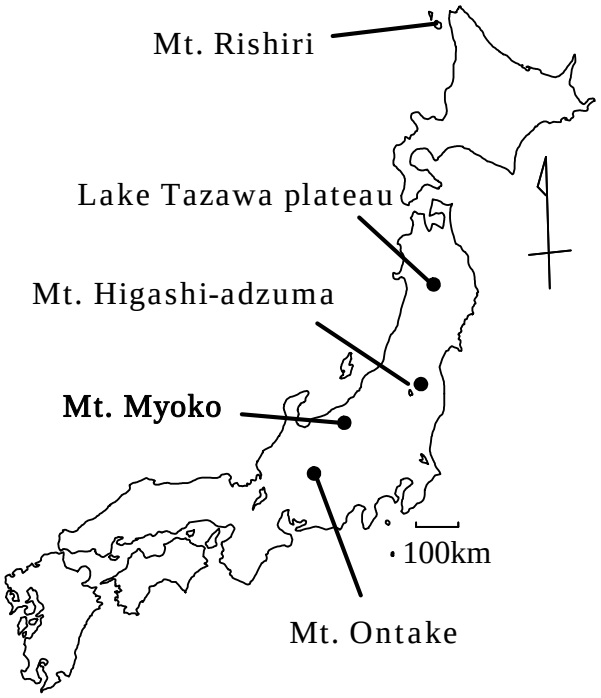

Fig. 2.1. Study areas of chapter 2.

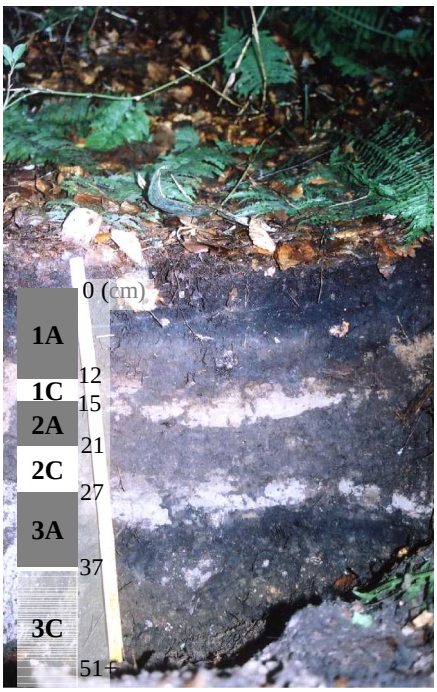

Fig. 2.2. Profile of Mt. Myoko soil. Picture is taken from Watanabe *et al*. (2007).

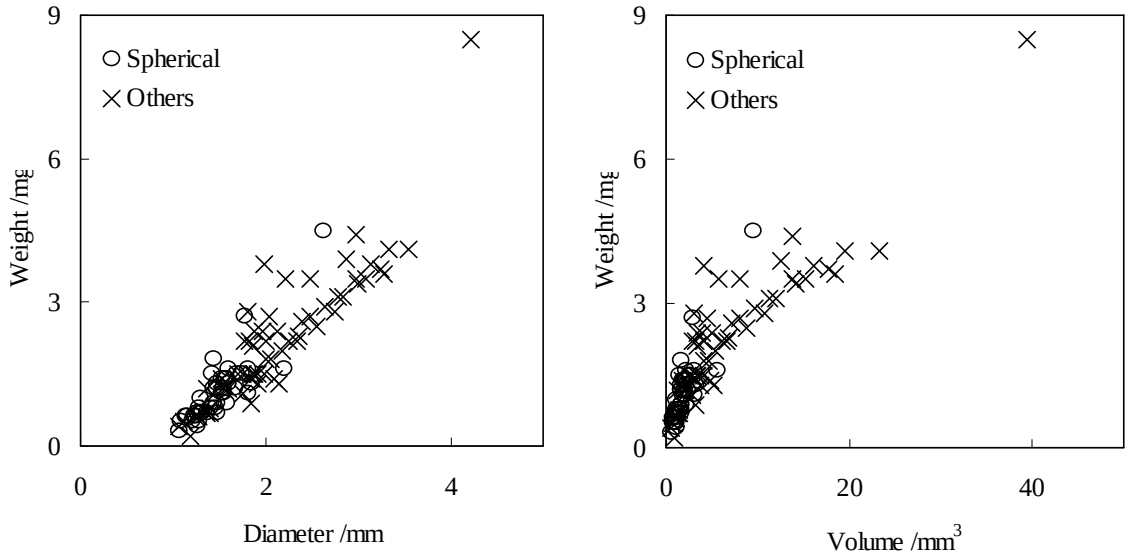

Fig. 2.3. Relationships between diameter, volume and weight of sclerotia. Opened circle (○) indicates spherical sclerotia and cross mark (×) indicates sclerotia in other features.

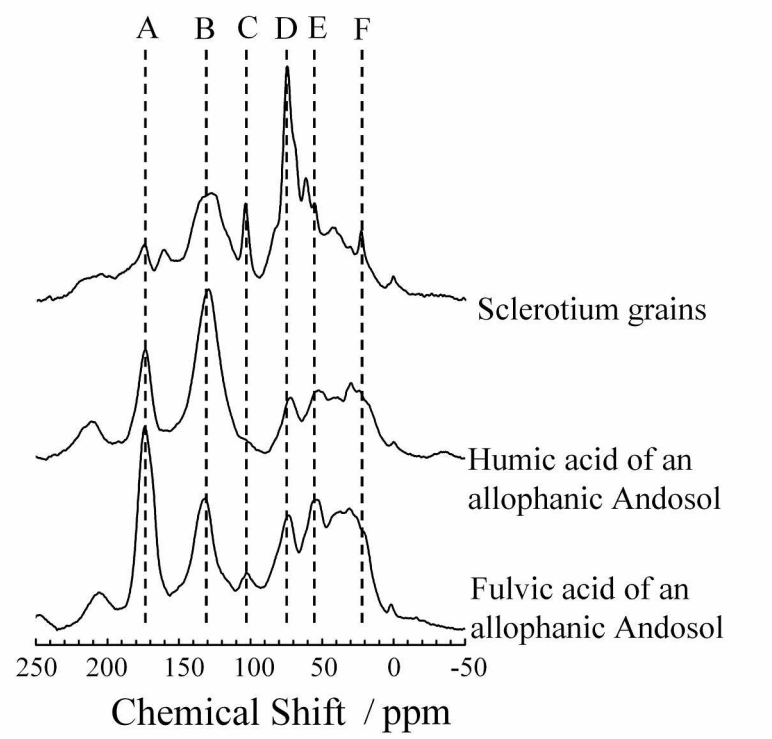

Fig. 2.4. Solid-state CPMAS ^{13}C NMR spectra of sclerotia, humic acid (HA) and fulvic acid (FA) of an allophanic Andosol. *Humic and fulvic acids were prepared from the Ib-G5 soil of Hiradate *et al*. (2004).

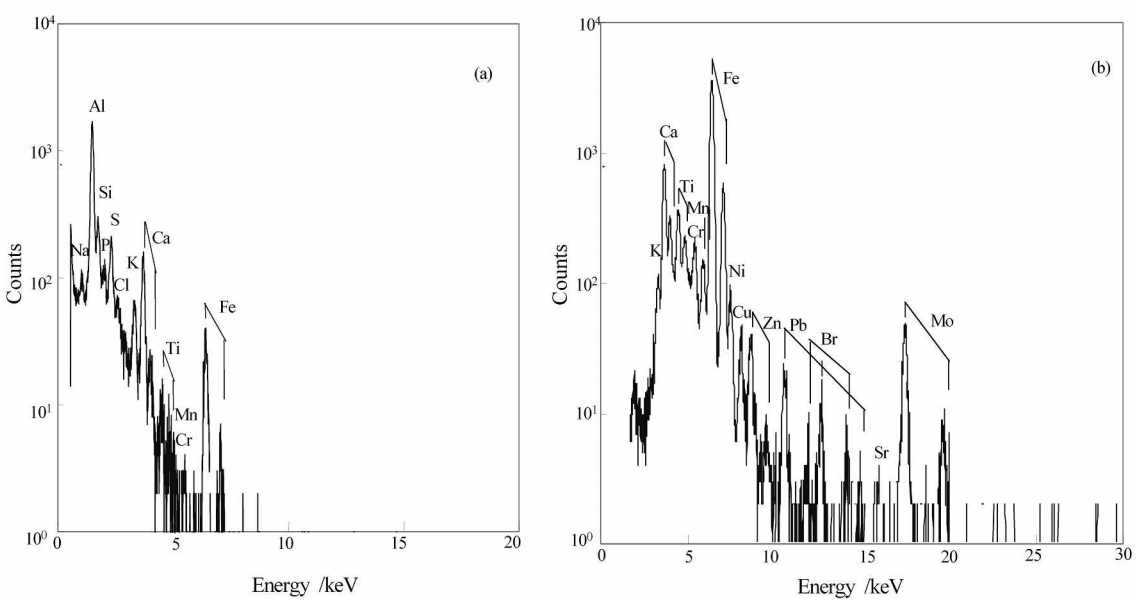

Fig. 2.5. PIXE spectra of sclerotia in (a) low and (b) high energy regions.

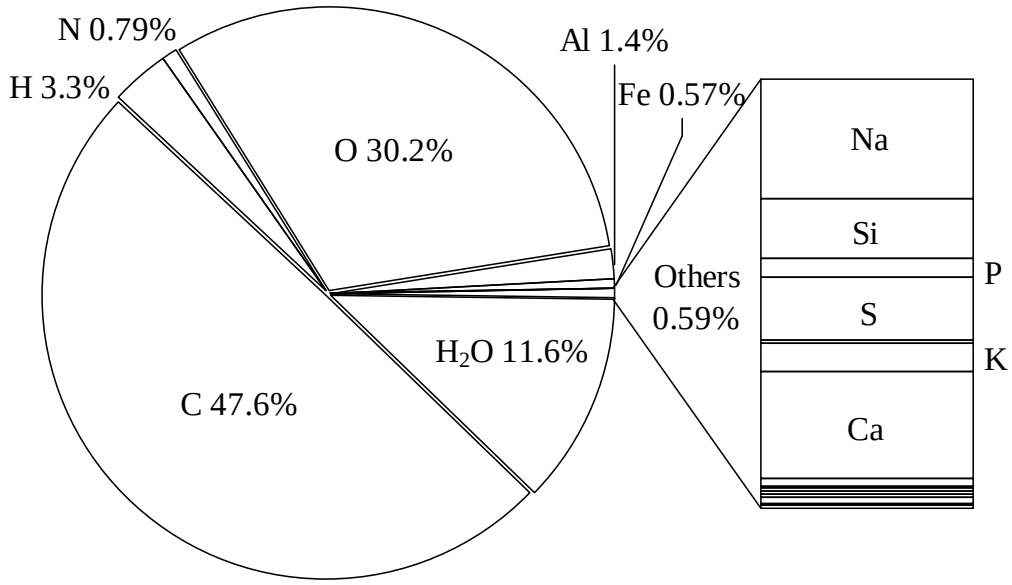

Fig. 2.6. Elemental composition of Myoko sclerotia by analyses of CHNO and trace element.

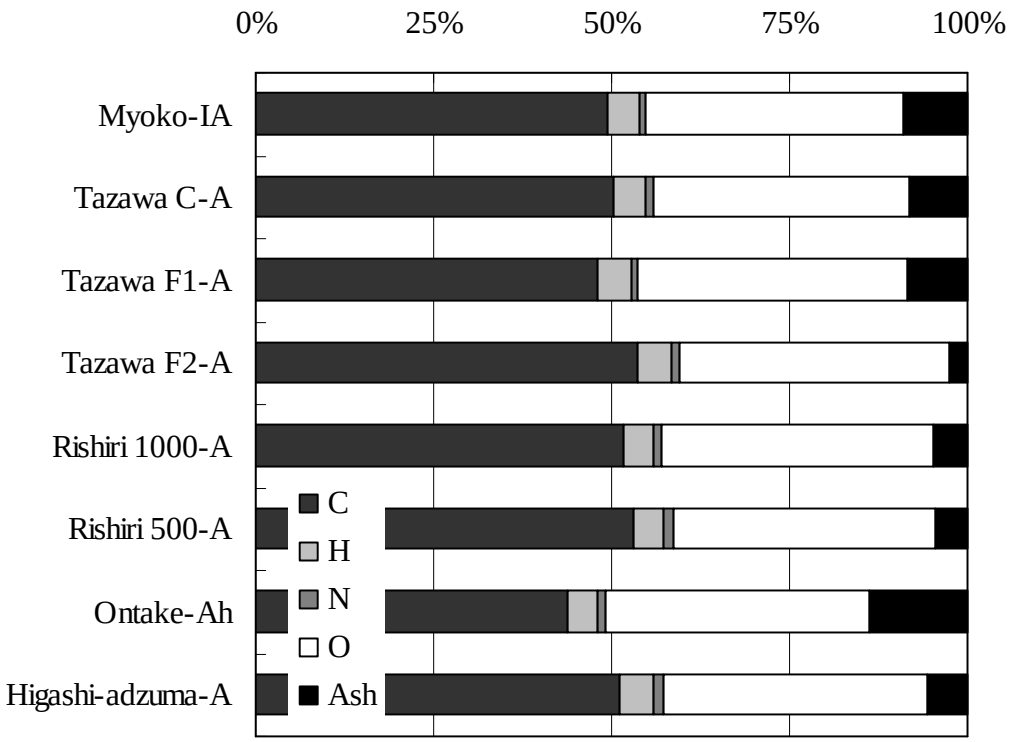

Fig. 2.7. Variance of CHNO and ash composition of sclerotia from surface soils.

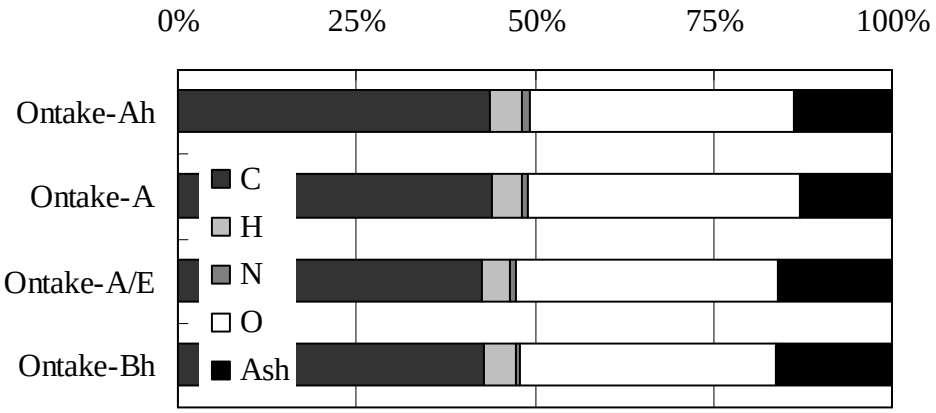

Fig. 2.8. Variance of CHNO and ash composition of sclerotia from Ontake profile.

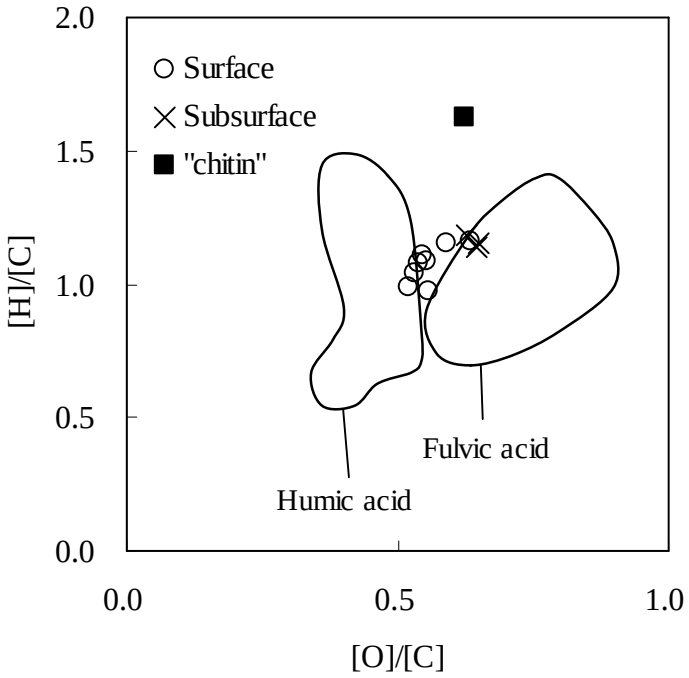

Fig. 2.9. The atomic ratio of O and C ([O]/[C]), and H and C ([H]/[C]) of sclerotia, chitin, humic and fulvic acids. The data of humic and fulvic acids were referred from Kumada (1987).

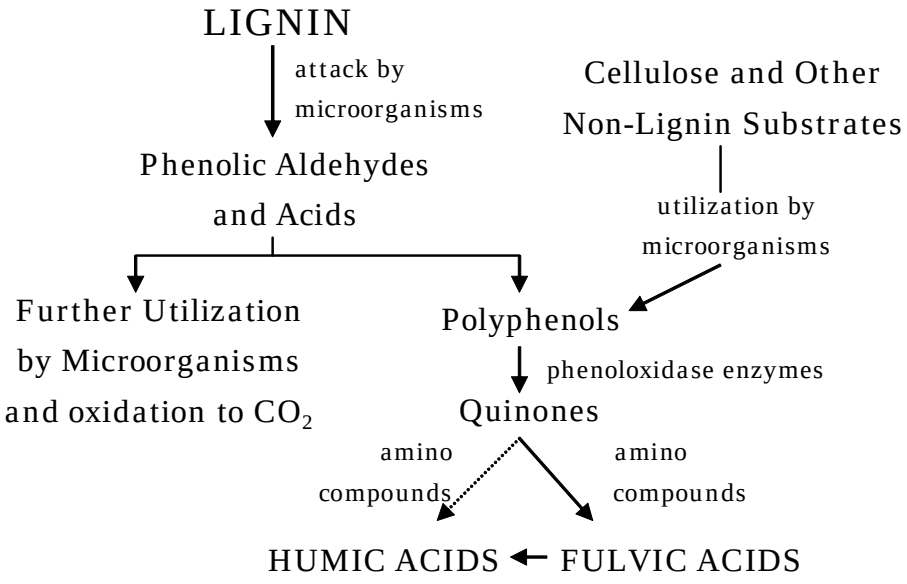

Fig. 2.10. “Polyphenol theory” of humus formation (Stevenson, 1994).

Chapter 3

Interaction and contribution to soil aluminum and iron

3.1 Introduction

As described in chapter 2, sclerotia contain Al and Fe, and these ashes content were likely to reveal their soil-environmental conditions. From the studies in Japanese and German forest soils, it was notified that the specific ratio of active Al in surface and buried A horizons, $Al_p/Al_o>0.5$, regulated the distribution of sclerotia, and the relationship between the mean weight of grains and the content of exchangeable Al was highly positive (Watanabe *et al*., 2002; 2004b). The role of the grains as persistent organic component in forest soils is assumed to have relation with toxic metal behaviour in soils.

As shown in Fig. 3.1, two types of sclerotia are differentiated by their internal features. First type is the grains collected from Andosols, Cambisols, or Luvisols, which usually do not have any part of medulla or non-melanized pseudoparenchymatous, and their color is blackish or dark brownish. Second type is the grains from Podzols, having yellowish or reddish transverse wall that suggests Fe concentration in the grain. The objective of this chapter is to elucidate the interaction and contribution of sclerotia to soils by determination of Al and Fe characteristics in grains collected from Japanese and German forest soils.

3.2 Materials and methods

Soils and sclerotia from forest soils in Japan, and in Harz Mts., Germany were used in this study (Fig. 3.2). Soils were kept dried in air for two weeks and then preserved in plastic bags. Sclerotia in soil samples were floated in pure water, carefully picked up from soils using tweezers and kept under air-dried condition. Contents of sclerotia in soils (sclerotial biomass) were obtained based on the weight (SG_w, mg g^{-1}) and the count (SG_c, count g^{-1}) in each soil samples. Almost all sclerotia from air-dried soils floated in water in this experiment. These floated sclerotia were considered to include both "live" and "dead" sclerotia, differentiated by Trappe (1969). The total C (T-C) and N (T-N) contents of soils were measured by the dry combustion method using a NC-analyzer (NC-80, Sumica Chemical Analysis Service Ltd., Tokyo). The value of the soil pH(KCl) were measured by the glass electrode method in the suspension mixture of soil with a 2.5 times volume of 1 M KCl. Quantitative analysis of acid oxalate extractable Al and Fe (Al_o, Fe_o) was carried out by the selective dissolution method (Blakemore *et al*. 1987). The content of exchangeable Al (Al_{Ex}) and Fe (1 M KCl-Fe) was obtained on the extract with 1 M KCl according to the method of Blakemore *et al*. (1987).

The mean contents of Al and Fe in sclerotia were determined using ICPS-8100 (Shimadzu Corp, Kyoto). Approximately 1 mg of powdered sample was placed in a beaker (5 ml) with 1 ml of ultra pure nitric acid (HNO_3) for about 5 minutes in an oven at 120 °C. Then 1 ml of 30% (v/v) hydrogen peroxide (H_2O_2) solution was added to the residue and was heated at < 100 °C for 2 minutes until almost evaporated, whereupon 1 ml of 0.1 M HNO_3 was added. Finally, the solution was again evaporated almost to dryness at 130~140 °C. The residue was dissolved in 1 ml of 0.1 M HNO_3.

Transmission electron microscopy (TEM) -EDX analysis was carried on fractions picked up from the internal structure of the grain from Mt. Higashi-adzuma using a JEM-2010F

(200keV, JEOL Ltd., Tokyo) electron microscope. Sclerotia from each soil were sliced into half pieces using a sterilized knife to observe the morphological features of the internal of the grains using a scanning electron microscope SEM (S-800, Hitachi, Tokyo), and to determine the difference of elemental composition of Al and Fe (carbon free) between the cutting surface (wall) and the internal structure (internal) using an energy dispersive X-ray spectrometer (EDS; EDAX PV9900I, Philips). The results of SEM-EDS are discussed by weight ratio of Fe/Al.

3.3 Identification of Fe-rich sclerotia and absorption of free Al and Fe in sclerotia

From TEM-EDX analysis on the internal structure of the grain from Mt. Higashi-adzuma, Al and Fe (1~12%) were determined with high content of carbon (65~85%) in the cell wall (Fig. 3.3). This fact confirms Al and Fe absorption in sclerotial cell wall is occur in process of biosynthesis. As a high concentration of Al is detected in cortex cell wall of ectomycorrhiza (Brunner and Frey, 2000; Brunner,2001), the absorption of Al in the cell wall of ignited grains suggests the grains themselves as biosynthetic product of *C. geophilum* responsible for Al retention.

Table 3.1 shows the results of chemical composition (weight ratio of Fe/Al) of wall and internal part of sclerotia, and 1 M KCl-Fe/Al_{Ex} ratio in soils. Weight ratio of Fe/Al of wall part of sclerotia ranged 0~0.77, and relatively high ratio of Fe/Al of internal part ranged 0.01~6.51. 1 M KCl-Fe/Al_{Ex} ratio in soils ranged 0~0.49. Further support for “absorption of Al and Fe” can be provided by the positive correlation between Fe/Al ratio of wall and 1 M KCl-Fe/Al_{Ex} ratio (Fig. 3.4). The absorption of Fe and Al ions in grains may occur with biosynthetic process of cell wall in equilibrium with the concentration of exchangeable Al and Fe ions in soil. This fact also suggests that sclerotia are likely to be formed in soils, where submerged with soil water. This hypothesis will harmonize with the fact that sclerotia are

formed in fantastically spherical feature.

As noted in the introduction of this chapter, sclerotia reveal two types of differentiated internal features. Since such differences were likely to be caused by their chemical compositions, relationship between Fe/Al ratio of internal and wall part are compared in Fig. 3.5. Sclerotia with Fe/Al ratio<1 showed a strong correlation between internal and wall parts of the grains. On the contrary, such correlation could not be recognized for sclerotia with high Fe/Al ratio in internal part (Fe/Al ratio of internal > 1). This fact suggests a possibility to define as “Fe-rich” sclerotia. The cause of such Fe concentration occurring in sclerotia is not well understood, but some consideration will be provided in chapter 4 and 5.

3.4 Regional change of Fe/Al ratio in sclerotia

In Mt. Rishiri and Harz Mts., soils and sclerotia were taken from different elevation. Fig. 3.6 and 3.7 shows the Fe/Al ratio of wall and internal part of sclerotia from Mt. Rishiri and Harz Mts. Sclerotia which have inhollow structure from Rishiri A, B, and Elend, Brandhai, Oderteich showed low ratio of Fe/Al both in wall and internal part. However, gradual increase of Fe/Al ratio was recognized in sclerotia from higher region. On the contrary, sclerotia with hallow structure revealed high Fe/Al ratio in internal part. Hollow sclerotia from Rishiri C,D and Oderteich could be recognized as “Fe-rich” sclerotia. This fact means high Fe concentration occurs in internal part of hollow sclerotia from higher elevation. In other words, Fe-rich sclerotia (Fe/Al ratio of internal > 1) distribute in Podsols of Mt. Rishiri and Harz Mts. This fact confirms that the chemical composition of sclerotia is strongly influenced by soil-environmental condition.

3.5 Contribution of sclerotia Al and Fe to the active Al and Fe in soils

Table 3.2 shows the results of mean content of Al and Fe in sclerotia, and SG_w and SG_c. The highest SG_w (2.4 mg g^{-1}) was observed in buried 3A horizon at Mt. Myoko, and the highest SG_c (34.8 count g^{-1}) was observed in surface horizon at Mt. Higashi-adzuma. The mean contents of Al and Fe ranged 0.26~2.98 wt %, and 0.16~5.74 wt %, respectively. As shown in Fig, 3.8, the mean contents of Al+Fe in sclerotia were strongly correlated with mean ash contents in sclerotia measured by analysis on CHNO composition in chapter 2. This fact confirms the analytical method on Al and Fe contents in sclerotia in this study is reliable, and also confirms that the characteristics of ashes in sclerotia can be explained by Fe and Al.

By multiplying SG_w and mean contents of Al+Fe, the contents of Al and Fe in the sclerotial form (i.e. contained in sclerotia) in soils (Al_{SG} and Fe_{SG} g kg^{-1}) can be estimated. As shown in Fig. 3.9, the amount of Al and Fe in sclerotial form had a slight correlation with active Al and Fe (Al_o+Fe_o). In this study, maximum contribution of Al_{SG}+Fe_{SG} to Al_o+Fe_o was 0.38% at the highest. However, such correlation in different dimension also confirms that the chemical composition of sclerotia is strongly influenced by status of active Al and Fe in soil -environment, and the existence of sclerotia have possible impact to interact with soil chemical properties. From these results, the characteristics of sclerotia are expected to become an indicator of soil chemical properties and fungal activities.

3.6 Conclusion

The ratio of Fe/Al in wall part of sclerotia had relation with the ratio of exchangeable Al and Fe in soils. Sclerotia were likely to be formed in soils, where submerged with soil water. The chemical composition of sclerotia was strongly influenced by soil-environmental condition.

Consequently, it was considered that the absorption, or retention of Al and Fe ions together with C by biosynthetic process may have a role to reduce phyto-toxic Al and Fe in low pH soils. Al_{SG} and Fe_{SG} (g kg^{-1}) were defined as Al and Fe in the form of sclerotia. The content of Al_{SG}+Fe_{SG} had slight correlation to the content of active Al and Fe in soils, and chemical composition of sclerotia was suggested to be strongly influenced by status of active Al and Fe in soil -environment, and the existence of sclerotia were considered to have possible impact to interact with soil chemical properties. In addition, the characteristics of sclerotia were expected to become an indicator of soil chemical properties.

Table 3.1. Results of chemical composition (weight ratio of Fe/Al) of wall and internal part of sclerotia, and 1 M KCl-Fe/Al_{Ex} ratio in soils.

Site	Horizon	Sclerotia		Soil
		Wall Fe/Al	Internal Fe/Al	1MKCl-Fe/Al_{Ex}
Rishiri A	A	0.21	0.18	0.20
Rishiri B	A	0.22	0.84	0.18
Rishiri C	A	0.17	1.28	0.19
Rishiri D	A	0.32	5.37	0.21
Akita (C)	A	0.12	4.87	0.12
Akita (B2)	A	0.07	0.18	0.16
Ontake	A1	0.76	3.59	0.21
		0.01	0.19	0.10
	A2	0.00	0.17	0.08
	A/E	0.02	0.01	0.05
	Bh	0.01	0.01	0.02
	Bw	0.01	0.04	0.04
Myoko	1A	0.01	0.02	0.01
		0.01	0.04	0.00
Jumonji	E	0.24	6.44	0.14
Ramberg	A	0.08	0.19	0.04
Brandhai	A	0.28	0.33	0.40
Elend	A	0.40	0.39	0.24
Oderteich	A	0.43	6.51	0.49
		0.77	0.89	0.49

Table 3.2. Results of mean content of Al and Fe in sclerotia, and sclerotial biomass based on weight and count (SG_w and SG_c) in unit weight soils.

Site	Horizon	Soil		Sclerotia		
		SG_w	SG_c	Al	Fe	Al+Fe
		mg g^{-1}	count g^{-1}	wt %		
Rishiri A	A	0.6	1.3	0.24	1.35	1.58
	AB	0.2	0.5	0.94	5.00	5.93
Rishiri C	A	0.2	0.3	0.72	0.74	1.45
	AB	0.1	0.2	2.59	5.74	8.33
Akita (C)	A	0.8	0.9	1.40	1.10	2.50
Akita (B1)	A	0.2	0.6	2.51	0.63	3.14
Akita (B2)	A	0.4	0.7	0.26	0.33	0.59
	AB	1.5	6.8	2.31	0.91	3.22
Higashi-adzuma	A	2.2	34.8	0.97	1.14	2.11
Ontake	A1	2.1	12.0	2.37	3.64	6.01
	A2	1.3	9.3	2.98	3.29	6.27
Myoko	1A	1.3	0.8	1.65	0.51	2.16
	2A	1.7	0.8	1.58	0.16	1.74
	3A	2.4	2.2	2.39	0.29	2.67

Fig. 3.1. Two types of internal structure of sclerotia from Myoko soil (left) and Higashi-adzuma (right).

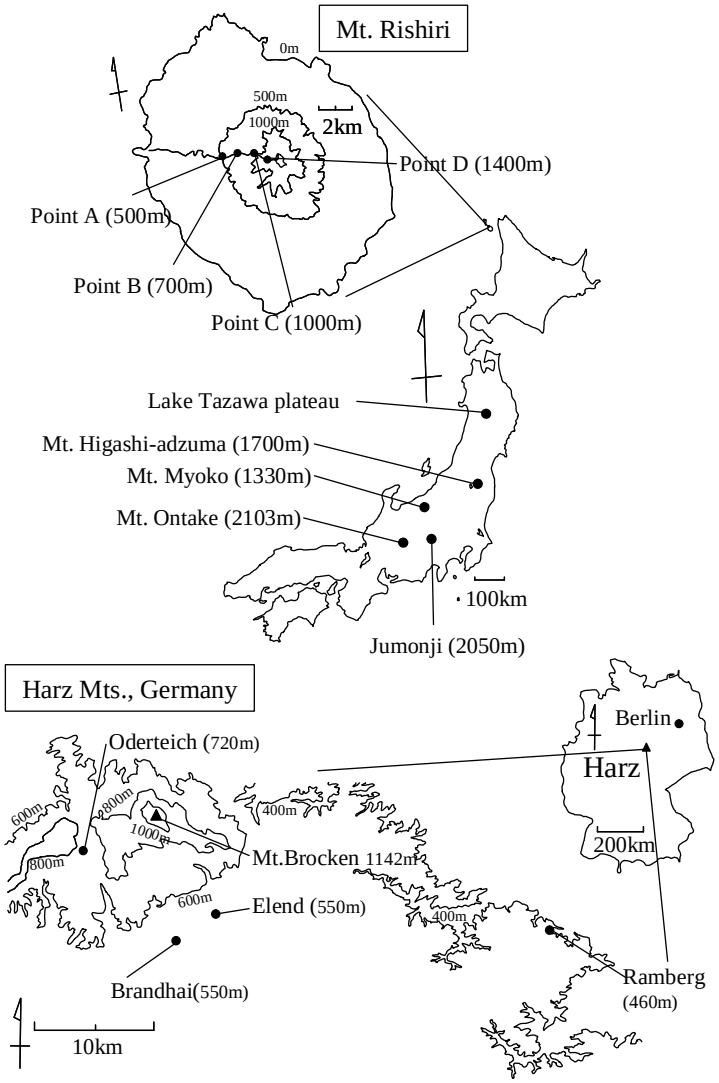

Fig. 3.2. Study areas of chapter 3.

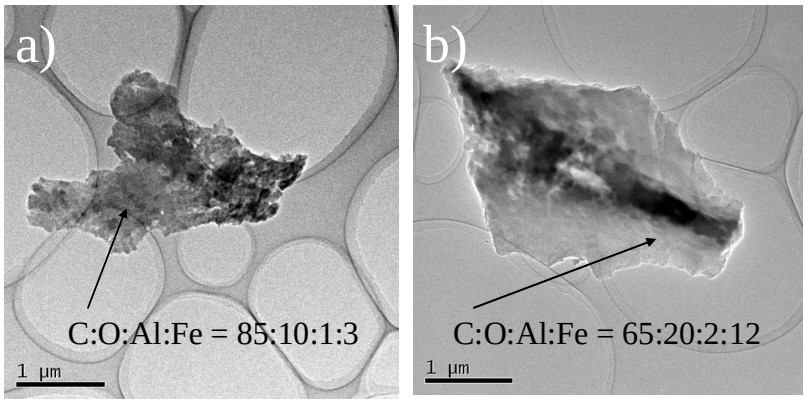

Fig. 3.3. Results of TEM-EDX analysis on cell wall of sclerotium.

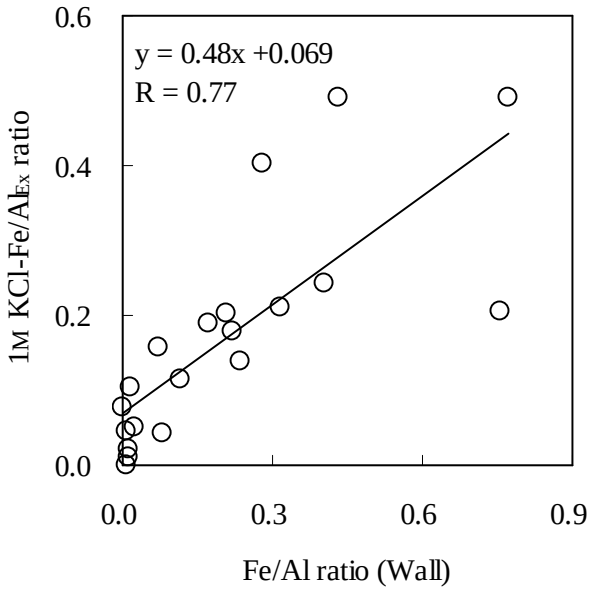

Fig. 3.4. Relationship between weight ratio of Fe/Al of wall part of sclerotia and 1M KCl-Fe/Al$_{Ex}$ ratio in soils.

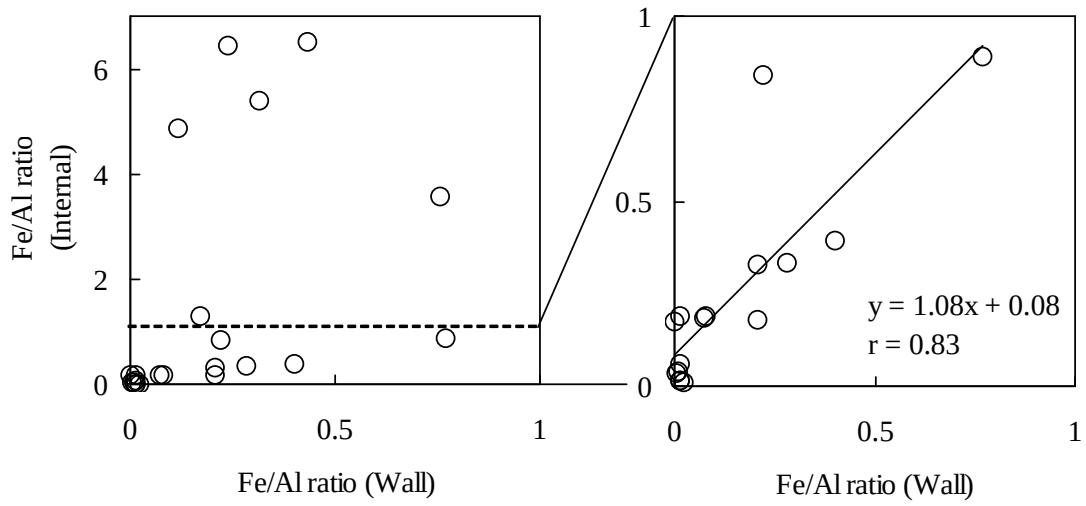

Fig. 3.5. Relationship between weight ratio of Fe/Al of wall and internal part of sclerotia.

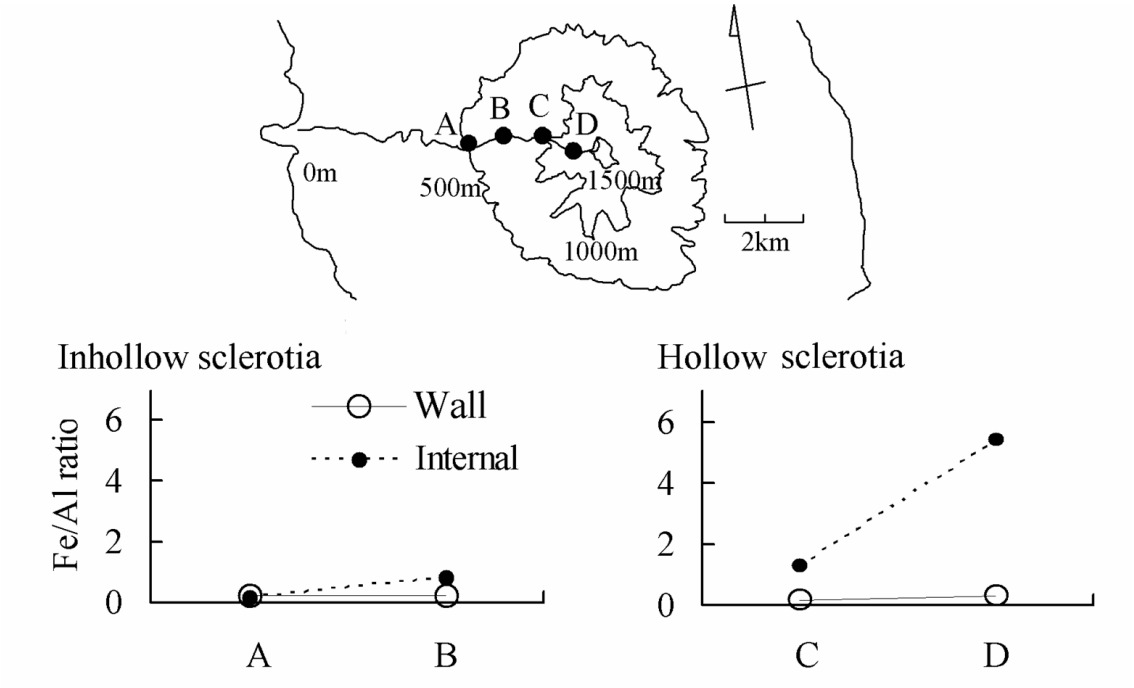

Fig. 3.6. Weight ratio of Fe/Al of wall and internal part in sclerotia from Mt. Rishiri.

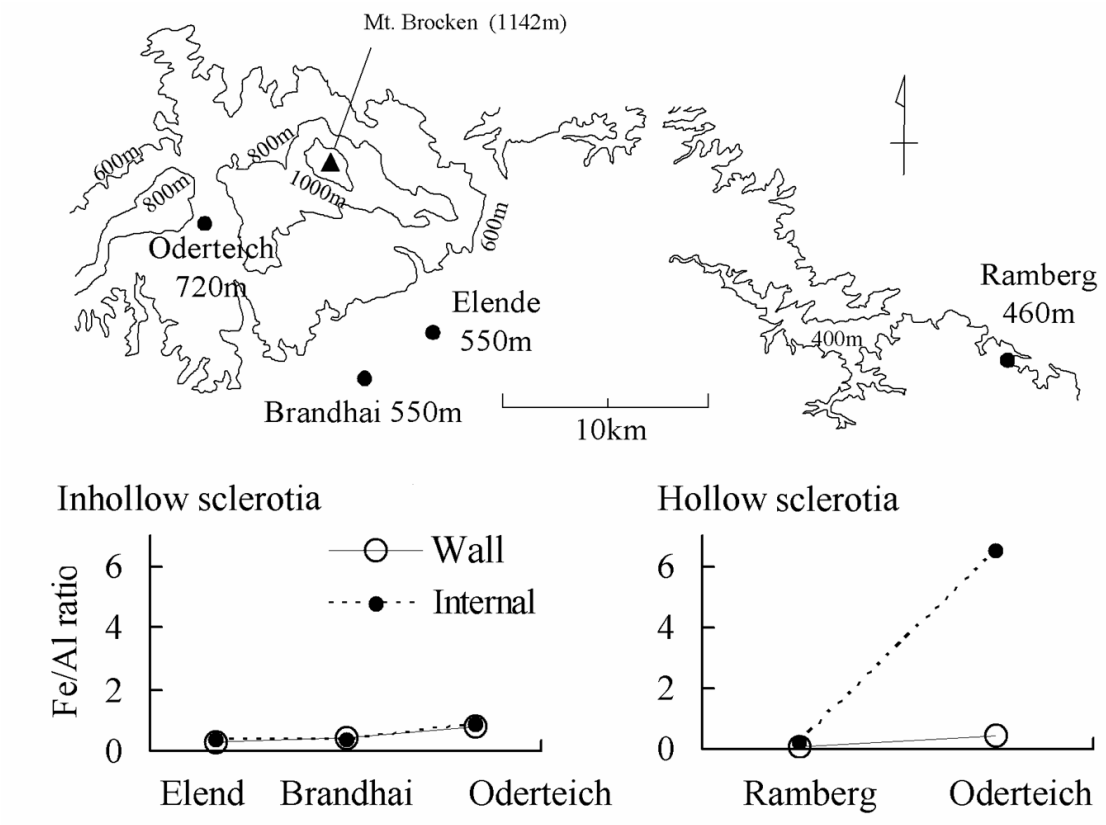

Fig. 3.7. Weight ratio of Fe/Al of wall and internal part in sclerotia from Harz Mts.

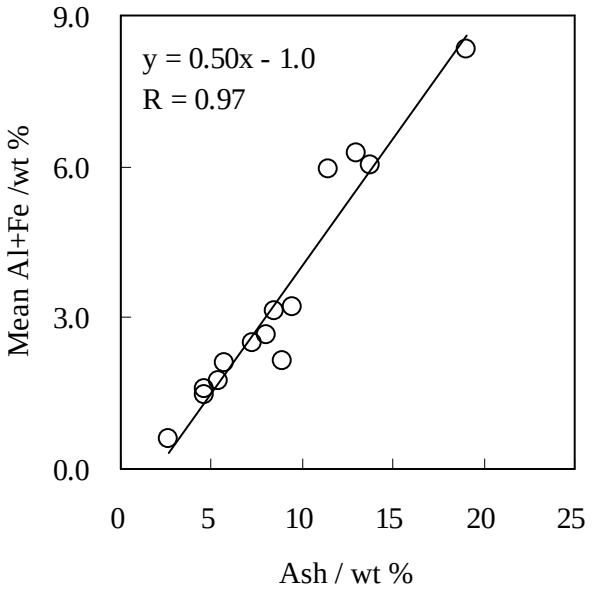

Fig. 3.8. Relationship between ash content measured with CHNO composition in chapter 2 and Al+Fe contents in sclerotia.

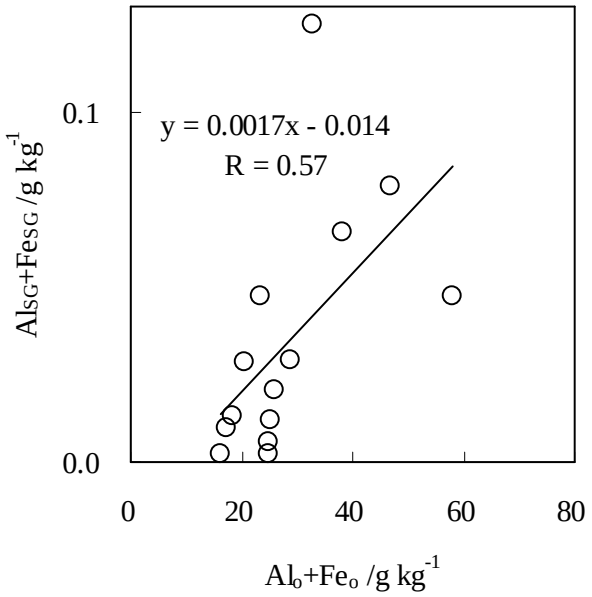

Fig. 3.9. Relationship between Al_o+Fe_o and $Al_{SG}+Fe_{SG}$ in soils.

Chapter 4

Spatial distribution and regulating factor of formation of sclerotia in low pH forest soils

4.1 Introduction

Trappe (1964) reported a worldwide distribution of *C. geophilum* by observation of its mycorrhiza in temperate and arctic-alpine climatic zones. Kumada and Hurst (1967) observed distribution of sclerotia in several British and Swedish Podsols. By investigation on the distributions of sclerotia in soil profiles of Japanese Andosols and German Podsols, Braunfahlerde and Brown Podsols, Watanabe *et al*. (2002; 2004b) concluded that formation of sclerotia was regulated by the content of exchangeable Al and the status of active Al in the soil, regardless of soil type. However, relatively few studies have been reported on spatial distribution of sclerotia in soils. As sclerotia exist in soils as one of significant soil organic component, their distribution in soils has to be clarified.

In this chapter, spatial distribution of sclerotial biomass in surface soil were examined in forest with single stand of *Picea abies* to clarify dynamics of sclerotial formation. The precise examination in large-scale is expected to elucidate the role of sclerotia as organic component in forest soils and interaction with soil chemical properties.

4.2 Materials and methods

The Harz Mts., located in central Germany, are 90 km long and 30 km wide. Mt. Brocken is the summit of Harz Mts. and the elevation is 1142 m. Paleozoic rock appears close to the surface and is covered with detrital accumulation, which was developed during the Pleistocene under periglacial conditions. This cover of debris contains weathered bedrock and wind-borne sediment like loess (Haasse *et al*., 1989). The climate of Harz Mts. is recognized as subatlantic climate to sub continental climate. Mainly due to Luv-Lee effect, the annual total precipitation in the Harz Mts. area decreases from the west (>1000 mm) to the east (<600 mm) and the highest regions are observed around Mt. Brocken (approximately 1700 mm). On the contrary, the annual mean temperature increase from the west (3~4 ºC) to the east (7~8 ºC) and Podzols dominates in higher regions above 600 m. According to Watanabe *et al*. (2004b), relatively large amount of sclerotia was observed in Elend. The predominant mineral in A horizon of the Elend soil (Braunfahlerde) was interstratified smectite-vermiculite layers and showed higher Al^{3+} content in spite of higher pH values (4.4 in H_2O, 3.0 in KCl) compared to Oderteich soil (Podzols) located in higher region (3.8 in H_2O, 2.0 in KCl). Humification degree of Elend soil was higher than Oderteich soil for both free and combined form humic acid (HA). The types of HA were determined as P and A /B for free HA and combined HA, respectively. More than 90% of the extracted HA was in free form. The SiO_2/R_2O_3 ratio of this profile was similar to Oderteich soil, while the content of extractable Al, Fe and Si was higher than Oderteich soil.

According to the above basic knowledge, study area was selected around Elend to examine the spatial distribution of sclerotia in low pH soils. Topographical measurement, investigation of floor vegetation and surface soil sampling were carried out in *Picea abies* forest near Elend and Königshütte (51°43~44'N, 10°42~45'E) in Harz Mts., Germany in late May 2005 (Fig. 4.1 and 4.2). The investigation was conducted for 7 lines in 5 sites. Angle of every 1

m was measured along 7 lines of length from 10 to 114 m, and surface soil samples (approximately 0~5 cm in depth) were collected at 86 points along the measurement line for the following soil analyses.

Sclerotia were carefully picked up from air-dried and homogenized surface soil samples using tweezers and kept under air-dried condition. 10~30 g of air-dried soil were stirred after adding 500 ml of distilled water to collect all sclerotia from the float of the suspension. The air-dried sclerotia mostly floated in water. The counts of sclerotia were obtained by following 6 divisions of their diameter range (0.2~0.5, 0.5~1.0, 1.0~1.5, 1.5~2.0, 2.0~2.5, and 2.5~3.0 mm). Sclerotial biomass was defined by weight (SG_w) and count (SG_c) of air-dried sclerotia per air-dried soil.

Chemical analyses were carried out on air-dried soil samples passing through a 0.5 mm sieve for the total C and N contents and a 2.0 mm sieve for other analyses. Content of fresh organics (plant roots, leaves, and barks) was measured by passing through a 2.0 mm sieve. The values of the soil pH in H_2O and KCl were measured by the glass electrode method in the suspension mixture of soil with a 5 times volume of H_2O and 1M KCl, respectively. The content of exchangeable Al (Al_{Ex}) was obtained for the extract with 1M KCl according to the method of Blakemore *et al*. (1987). The extract was titrated by 0.01M NaOH, and then adding 4 ml of NaF, the content of Al_{Ex} was estimated by titration using 0.01M HCl. The content of Al extracted by sodium pyrophosphate (Al_p) by shaking for 16 hours was measured using an inductively coupled plasma spectrometry (ICPS-1000IV, Shimadzu Corp, Kyoto). The total C (T-C) and N (T-N) contents were measured by dry combustion method using a NC-analyzer (NC-80, Sumica Chemical Analysis Service Ltd., Tokyo). Melanic index (MI) and Pg index (PI) were obtained by the procedure proposed by Honna *et al*. (1988) and Yamamoto *et al*. (2000). Colorimetric properties of the 1M NaOH extracted humic acids, which represent for free form type HA, were measured by using a UV spectrophotometer (UV 2400, Shimadzu Corp, Kyoto). Absorbance values at 450, 520, 600 and 610 nm were measured, and K450/K520 and K610/KA600 were

calculated to obtain MI and PI, respectively.

Air-dried sclerotia were sliced into half pieces using a sterilized knife to observe the morphological features and chemical composition of transverse wall. For this operation, grains were selected from points B-5, C-5, E-8, that have different soil pH(KCl) value and Al_{Ex} content. Chemical composition was obtained for these samples using a scanning electron microscope (SEM) and energy dispersion X-ray spectrometer (EDS) (JSM-6490LA, JEOL Ltd, Tokyo, accelerating voltage of 15 eV).

4.3 Diversity of sclerotial biomass

4.3.1 Results of Micro-topography and sclerotial biomass

Fig. 4.3(1) and (2) describes the distribution of sclerotial biomass (SG_w and SG_c) along the measured lines in sites A~E. Table 4.1 represents average, maximum, and minimum content in each site. Averages of sclerotial biomass (SG_w and SG_c) through all points were 0.54 g kg^{-1} and 1.3 count g^{-1}, respectively.

Site A and B is a side slope and a crest slope, respectively. The maximum sclerotial biomass in site A was at point A-2: 2.0 g kg^{-1} of SG_w and 2.1 count g^{-1} of SG_c. The maximum sclerotial biomass in site B was at point B-2, near the end of side slope: 1.1 g kg^{-1} of SG_w and 3.0 count g^{-1} of SG_c. There was no sclerotia at the end of the opposite side slope of site B. This point had no *Picea abies* stands but had grass coverage. Site C is a transverse of lower side slope and bottomland across a river. Sclerotia constantly distributed in the bottomland. Three parallel transverse lines of site D: D1~D3, were defined to cross the watercourse as head hollow. Point D2-7 showed the maximum SG_c and D3-9 had the maximum SG_w, which were 7.8 count g^{-1} and 2.8 g kg^{-1}, respectively. Five points in site D contained no sclerotia. These points were

relatively flat and had plenty of crude organics. Site E is a current head hollow in tangential to the sideslope line. Point E-2 showed the maximum sclerotial biomass at site E: 5.4 g kg^{-1} by SG_w and 5.8 count g^{-1} by SG_c. Point E-9, near edge of a cliff, showed a higher sclerotial biomass compared to the adjacent points. The increase of sclerotial biomass at point E-14 might be caused by transportation of sclerotia from the cliff wall. As for site E, there was an exceptional point that had no sclerotia, while it is notable that sclerotia still existed at the wall of the cliff.

4.3.2 Results of evaluation of floor vegetation

Floor vegetation was evaluated by qualitatively intensity (0~3) and discerned to I: Lichens and Bryophyta (i: except Polytrichaceae; ii: Polytrichaceae); II: Pteridophyta; III: Monocotyledoneae (i: Agropyron; ii: others) IV: Dicotyledoneae (except *Trifolium repens*); and V: *Trifolium repens*.

Principal component analysis (PCA) was conducted with SPSS 10.0.5J (SPSS Japan Inc., Tokyo) to characterize the population of floor vegetation. First component (PC1: 23%) mainly explained vegetation II, IV, (V), and second component (PC2: 20%) mainly explained vegetation III, V, and third component (PC3: 19%) mainly explained vegetation I as shown in Table 4.2. Using PC1 and PC2, six types of floor vegetation (V0~V5) were categorized as shown in Fig. 4.4. V0 was poor in floor vegetation, and V2 was rich in Bryophyta and *Trifolium repens*. V4 and V5 were characterized by occupation of Monocotyledoneae and Dicotyledoneae, respectively. V1 and V3 were intermediates of V0/V2 and V0/V4, respectively. Fig. 4.5 shows the representative pictures of the six types (V0~V5) of floor vegetation.

4.3.3 Sclerotial magnitude

Sclerotia are considered to be resistant against microbial attack during the period of inactivity as well as to desiccation (Cochrane, 1958; Gray and Williams, 1971; Fox, 1986). Matsumoto and Tajimi (1988, 1990) reported that the formation size of sclerotia contribute a fungal strategy to adapt environmental changes in study of *Typhula* species. They considered that certain species in area with difficulty to forecast environmental changes tend to form smaller sclerotia with less reserve substances in active term, and they accommodate for every eventuality. In this study, the diameter of sclerotia ranged 0.2~3.0 mm. As previously mentioned in chapter 1, *C. geophilum* is considered as a highly heterogeneous species or a fungal complex representative of a broader taxonomic rank (LoBuglio *et al.* 1991). Under the assumption that different size of sclerotia are formed by different species, Shannon-Weiner diversity index for the size class (each 0.5 mm in diameter) of sclerotia were calculated according to the following equation,

$$H' = -\sum pi \log 10\ pi \qquad (4.1$$

where *pi* indicates the proportional SG_c for grains of each diameter. Fig. 4.6 shows the Shannon-Weiner diversity index of size class of sclerotia. From this result, it is suggested that the formation of larger sclerotia may imply formation of sclerotia in wider range of size. Wider size range of sclerotia will insure *C. geophilum* to survive persistently in any environmental eventuality.

As shown in Fig. 4.3(1) and (2), idiosyncratic distribution of sclerotial biomass is recognized in some investigated soils. Especially in the shoulder part of slope edge, a relatively high contribution of sclerotial biomass was recognized (A-2, B-2, D3-9, E-9). In such geographical position, soils can be easily confronted to erosion and *C. geophilum* seems to form large amount of sclerotia to survive. On the other hand, point D2-7 showed large number of small sclerotia. This fact may suggest that the small grains are ready to be easily diffused with

soil erosion by water.

Fig. 4.7 shows distribution of sclerotia described by the magnitude of SG_w for each size class of maximum diameter of sclerotia in concern with floor vegetation (PC 1 and 2). Contribution of SG_w tended to be high around V0 and V3, and large sclerotia tended to form only around V0 and V3. This fact may suggest that formation of sclerotia in large size and large amount is promoted under the lack of floor vegetation condition (V0) or vegetation with few Monocotyledoneae condition (V3). Shaw and Sidle (1982) reported that the ability of live sclerotia to survive for several years could provide sufficient inoculum to colonize host species effectively. In addition, Watanabe *et al*. (2001) showed a mycelium-like structure in central part of sclerotia, and incubation of *C. geophilum* and related species from sclerotia, expected to have ^{14}C age of several hundred years, have been performed (unpublished observation). Although the causes of existence of large sized sclerotia is not clear, it might be understood as abundant formation or less decomposition of sclerotia. Furthermore, this fact will support the "pioneering ability" which was reported by Trappe (1988). Quantitative analysis on root infection of *C. geophilum* or other mycorrhizal fungi will serve to clarify contribution to preservation of the species for longer time by formation of large sclerotia.

4.4 Regulating factor of formation of sclerotia

4.4.1 Results of pH, Al_{Ex}, T-C and C/N ratio

The results of soil pH, Al_{Ex}, T-C and C/N ratio are compiled in Table 4.3. The values of pH (H_2O) and pH (KCl) were 3.0~4.5 and 2.5~4.0, respectively. The average value of pH (KCl) was 3.7 for site B, which had a relatively high pH value among the 5 sites with total average of 3.1. Content of Al_{Ex} was higher in lower pH soils and the maximum content was 2.5

g kg^{-1}. Total average of Al_{Ex} content was 0.58 g kg^{-1}, but such acidity was not noticeable in site B. T-C was approximately 150~300 g kg^{-1}. As contents of crude organics in soil sample was high at site A and E, high T-C values (> 400 g kg^{-1}) obtained from site A and E had a possibility of containing crude organics in the analyzed soil samples. C/N ratio was 13~24.

4.4.2 SEM-EDS analysis on sclerotia

Fig. 4.8 shows the morphological features of sclerotia observed by SEM and spectra of EDS analysis, where target area of EDS are denoted by open squares (a~c). Values of pH(KCl) and content of Al_{Ex} of points B-5, C-5, and E-8 are 4.4, 3.2, 2.8, and 0.04, 0.91, and 2.49 g kg^{-1}, respectively. The ratio of Fe/Al by weight for B-5, C-5, and E-8 were 0, 1.6, and 3.8, respectively. Sclerotium from B-5 was characterized by concentration of Al. While sclerotia from lower pH soils tended to increase relative amount of Fe.

4.4.3 Aluminum and iron as regulating factor of formation of sclerotia

According to the investigation of 12 profiles in Harz Mts. (Watanabe *et al.*, 2004b), formation and development of sclerotia did not always have a close relationship with low pH but had a closer relationship to the content of Al_{Ex}. As Fig. 4.9 shows, the relationship between contents of Al_{Ex} and maximum size of sclerotia confirmed from this study. As the similar relation was observed in such limited forest area covered with homogeneous stands, it can be considered that the content of Al_{Ex} as a truly effective factor on accelerating formation of large sclerotia. Formation of large sclerotia can be interpreted as physiological response of *C. geophilum* in Al stress condition.

Fig. 4.10 shows a good correspondence between content of ergosterol in soil and sclerotial biomass in the ergosterol-rich soil profiles of Brandhai, Sorge, and Allrode (revised

from Watanabe *et al*., 2004b). However, such correlation was not found in the ergosterol-rich Heinrichsburg soil profile, in which content of Al_{Ex} was relatively low despite of low pH. Ergosterol represents living fungal biomass (Grant and West, 1986; Montgomery *et al*., 2000). As shown in Fig. 4.11, soils with higher fungal biomass tend to have higher C/N ratio. Fig. 4.12 shows that investigated soils in this study reveal to enlarge contribution of sclerotia in low pH (<3.5) and high C/N ratio (>19) conditions. These facts suggest that the activity of fungi promote formation of sclerotia, only in case of that *C. geophilum* have large contribution to fungal flora in low pH soils.

From the results of SEM-EDS analysis, sclerotia in low pH soil were likely to increase Fe content. Ferricrocin is known to an ectomycorrhizal siderophore of *C. geophilum* (Haselwandter and Winkelmann, 2002; Hoffland *et al*., 2004). Absorption of Al and Fe may be an evidence of activity of *C. geophilum* associated with siderophore. This fact harmonizes with microbial dissolution of Al and Fe from soil minerals studied on ectomycorrhizal fungus (Watteau and Berthelin, 1994).

4.5 Significance of sclerotia as a soil organic component

4.5.1 Results of Al_p, MI and PI

The results of soil Al_p, MI and PI are compiled in Table 4.4. The range and average of Al_p content were 0.16~6.3 g kg^{-1} and 2.1 g kg^{-1}, respectively. Al_p content was relatively high at site C (average 3.1 g kg^{-1}) and low at site D (average 1.7 g kg^{-1}). MI and PI values were 1.9~2.3 and 0.91~1.0, respectively. Lower MI value indicates higher degree of humification except in case of Pg rich soil, and higher PI value indicates higher content of “Pg”, the green pigment derived from fungal metabolites. Using indices of MI and PI, humic acid type is categorized

into the following 5 categories. Type A: PI ≤ 0.98 and MI ≤ 1.70; type B and P_0: PI ≤ 0.98 and 1.70 < MI < 2.00; type $P_{+\sim+++}$: PI > 0.98; type $P_{\pm}$ and Rp(1): 0.93 < PI < 0.98 and MI ≥ 2.00; type Rp(2): PI ≤ 0.93 and MI ≥ 2.00 (Yamamoto *et al*., 2000).

4.5.2 Contribution of sclerotia as a soil organic component in low pH soils

According to the result of MI and PI, soil samples in this study were categorized as type P± and Rp(1) or Rp(2), with few exceptions in type $P_{+\sim+++}$, type B and P_0 (Fig. 4.13). Kumada and Hurst (1967) assumed sclerotia of *C. geophilum* as the source of Pg. However, large contributions of sclerotial biomass in Pg rich soil were not confirmed. This obtained fact rather harmonized with Valmaseda and Martínez (1989) that reported the small contribution of existence of sclerotia for Pg content in soil. Consequently, Pg rich soils that lack sclerotia may be understood that Pg content are likely to be derived from activities of micro-organisms, but not from formation or existence of sclerotia.

As it is known that approximately 50 wt% of sclerotium consists of C, contribution of sclerotial C to T-C can be estimated from SG_w/T-C (%). The maximum contribution of sclerotial C to T-C was 1.0% and soils of type P± and Rp(1) tended to have relatively large contribution of sclerotial C. According to Kumada (1987a) and Kuwatsuka *et al*. (1978), intergradation from Rp(2) to Rp(1) is recognized as the process of humification. In this process, consumption of easily decomposed organic matter and increase of fungal protein may occur. Formation of sclerotia can be considered as a primitive phenomenon in humification process. Uchida (1987) reported the contribution of microbial C to Soil T-C in boreal *Picea mariana* (black spruce) forest in Canada (53°50’N, 105°30’W). They observed that percentage of microbial biomass C in the L, FH, A, E and B horizons were 4.2, 2.4, 1.0, 2.4 and 5.2%, respectively. Comparing to this fact, sclerotial C was equivalent to microbial C. C reserved as sclerotia by *C. geophilum* may contribute to enlarge mean residence time of C in subalpine forest soil.

According to Watanabe *et al*. (2001), the ^{27}Al MAS NMR spectrum assigned predominant state of Al contained in sclerotia as 6-coordinated Al, which suggested the presence of Al-humus complex. Soils investigated in this study had high concentration of phyto-toxic Al_{Ex}. Even though the content of Al_p and Al_{Ex} is under equilibrium depending on release rate (Mulder *et al*., 1989; Berggren and Mulder, 1995), binding with humic substances as humus complex may reduce the Al toxicity for plant roots. Fig. 4.14 can be interpreted to show the two different directivities for the status of active Al in the studies soils; one revealed by high binding ratio of Al_p ($Al_p - Al_{Ex}$) to T-C, and another by large sclerotial biomass. The value of high ratio of Al binding as humic substance results from relatively high Al_p and low T-C content, which may mainly due to humus synthesis by catalysis of inorganic components. However, from the aspect of Al retention, two types of biological process for reducing Al stress are suggested; one is the form action of Al-humus complex, and another is the form action of sclerotia. According to Watanabe *et al*. (2007), sclerotia remain in soils for several hundreds or thousand years as persistently structured organic component. Absorption of Al and Fe in sclerotia was considered as a Fe reserve for *C. geophilum* itself, and retention of phyto-toxic Al in form of biosynthesized humic substances. Although the Al content of sclerotia in soils is not so large, the phenomenon of Al retention by sclerotia may be enhanced in rhizosphere, where sclerotia have close contact to plant root to play a significant role as a soil organic component.

4.7 Conclusion

Role of sclerotia as soil organic component were discussed in this chapter by interpreting distributional properties of sclerotia that are remaining in soils. From significant relationships between sclerotial biomass, determined as weight and count of the grains, and soil chemical properties, it was clarified that the distribution of sclerotia was regulated by Al_{Ex}

content and fungal activities, and was more likely to playing a role towards formation of humus-metal complex in lower pH soils. Absorption of Al and Fe in sclerotia was considered as a Fe reserve for *C. geophilum* itself, and role of retention of phyto-toxic Al in form of biosynthesized humic substances.

Table 4.1. Results of sclerotial biomass (SG_w and SG_c).

	SG_w	SG_c
	mg g^{-1}	count g^{-1}
All data (n=86)		
Ave.	0.5 ± 0.7	1.3 ± 1.3
Max./Min.	5.4 / 0.0	7.8 / 0.0
Site A (n=9)		
Ave.	0.6 ± 0.6	1.1 ± 0.7
Max./Min.	2.0 / 0.0	2.1 / 0.1
Site B (n=12)		
Ave.	0.5 ± 0.3	1.6 ± 0.9
Max./Min.	1.1 / 0.0	3.0 / 0.0
Site C (n=13)		
Ave.	0.6 ± 0.5	1.5 ± 0.9
Max./Min.	1.5 / 0.0	3.1 / 0.4
Site D (n=32)		
Ave.	0.3 ± 0.5	1.1 ± 1.7
Max./Min.	2.8 / 0.0	7.8 / 0.0
Site E (n=20)		
Ave.	0.8 ± 1.2	1.2 ± 1.4
Max./Min.	5.4 / 0.0	5.8 / 0.0

Table 4.2. PC 1, 2 and 3 of vegetation analyzed by principal component analysis. Vegetation I: Lichens and Bryophyta (i: except Polytrichaceae; ii: Polytrichaceae); II: Pteridophyta; III: Monocotyledoneae (i: Agropyron; ii: others) IV: Dicotyledoneae (except *Trifolium repens*); and V: *Trifolium repens*.

	Principal component		
	1	2	3
Explained variance	23%	20%	19%
Vegetation I (i)	0.180	0.234	0.234
Vegetation I (ii)	-0.169	0.072	0.072
Vegetation II	0.739	0.003	0.003
Vegetation III (i)	0.131	0.483	0.483
Vegetation III (ii)	0.248	0.775	0.775
Vegetation IV	0.818	0.087	0.087
Vegetation V	0.507	-0.708	-0.708

Table 4.3. Results of pH (H_2O, KCl), Al_{Ex}, total carbon (T-C), total nitrogen (T-N) and C/N ratio.

	pH(H_2O)	pH(KCl)	T-C	T-N	CN ratio	Al_{Ex}
			g kg^{-1}			g kg^{-1}
All data (n=86)						
Ave.	3.9 ± 0.4	3.1 ± 0.5	269 ± 117	13.4 ± 4.9	19.5 ± 2.5	0.58 ± 0.38
Max./Min.	5.6 / 3.4	5.2 / 2.6	518 / 37	23.8 / 2.4	24.0 / 13.6	2.49 / 0.00
Site A (n=9)						
Ave.	3.9 ± 0.2	3.1 ± 0.2	303 ± 101	14.4 ± 4.3	20.8 ± 2.4	0.61 ± 0.30
Max./Min.	4.2 / 3.7	3.3 / 2.9	443 / 140	19.4 / 7.8	22.9 / 16.0	1.14 / 0.16
Site B (n=12)						
Ave.	4.4 ± 0.5	3.7 ± 0.6	237 ± 69	13.1 ± 3.2	18.0 ± 1.5	0.31 ± 0.22
Max./Min.	5.6 / 3.8	5.2 / 3.0	339 / 133	17.6 / 7.2	19.9 / 15.4	0.79 / 0.00
Site C (n=13)						
Ave.	4.0 ± 0.4	3.2 ± 0.5	198 ± 98	10.3 ± 4.3	18.7 ± 2.7	0.74 ± 0.34
Max./Min.	4.6 / 3.5	4.0 / 2.6	358 / 75	16.3 / 4.3	22.4 / 14.8	1.25 / 0.28
Site D (n=32)						
Ave.	3.8 ± 0.4	3.0 ± 0.4	332 ± 123	16.2 ± 4.7	19.9 ± 2.6	0.41 ± 0.26
Max./Min.	5.1 / 3.4	4.5 / 2.6	518 / 103	23.8 / 7.4	23.7 / 13.6	1.01 / 0.00
Site E (n=20)						
Ave.	3.9 ± 0.2	3.0 ± 0.2	219 ± 99	10.7 ± 4.2	19.9 ± 2.3	0.90 ± 0.45
Max./Min.	4.6 / 3.5	3.7 / 2.6	375 / 37	16.7 / 2.4	24.0 / 15.5	2.49 / 0.37

Table 4.4. Results of Al_p and value of melanic index (MI) and Pg index (PI).

	Al_p / g kg^{-1}	MI	PI
All data (n=86)			
Ave.	2.15 ± 1.05	2.15 ± 0.06	0.94 ± 0.02
Max./Min.	6.30 / 0.16	2.32 / 1.95	1.02 / 0.91
Site A (n=9)			
Ave.	1.81 ± 0.63	2.11 ± 0.02	0.95 ± 0.01
Max./Min.	2.64 / 1.16	2.15 / 2.07	0.97 / 0.93
Site B (n=12)			
Ave.	2.15 ± 0.95	2.13 ± 0.06	0.95 ± 0.02
Max./Min.	3.69 / 0.93	2.24 / 2.04	0.97 / 0.92
Site C (n=13)			
Ave.	3.09 ± 1.29	2.14 ± 0.09	0.94 ± 0.03
Max./Min.	6.30 / 1.57	2.32 / 1.95	1.02 / 0.91
Site D (n=32)			
Ave.	1.67 ± 0.99	2.17 ± 0.05	0.93 ± 0.02
Max./Min.	4.05 / 0.16	2.24 / 1.97	0.96 / 0.91
Site E (n=20)			
Ave.	2.43 ± 0.65	2.17 ± 0.05	0.94 ± 0.01
Max./Min.	3.58 / 1.48	2.26 / 2.08	0.97 / 0.91

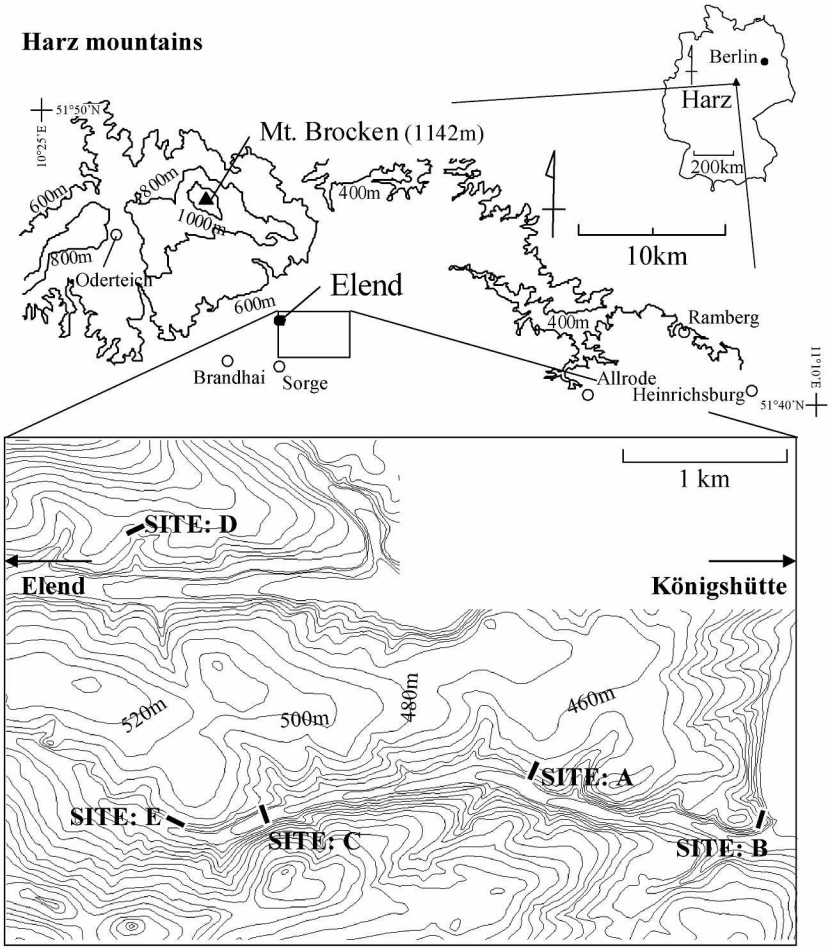

Fig. 4.1. Location map of study areas in Harz Mts., Germany.

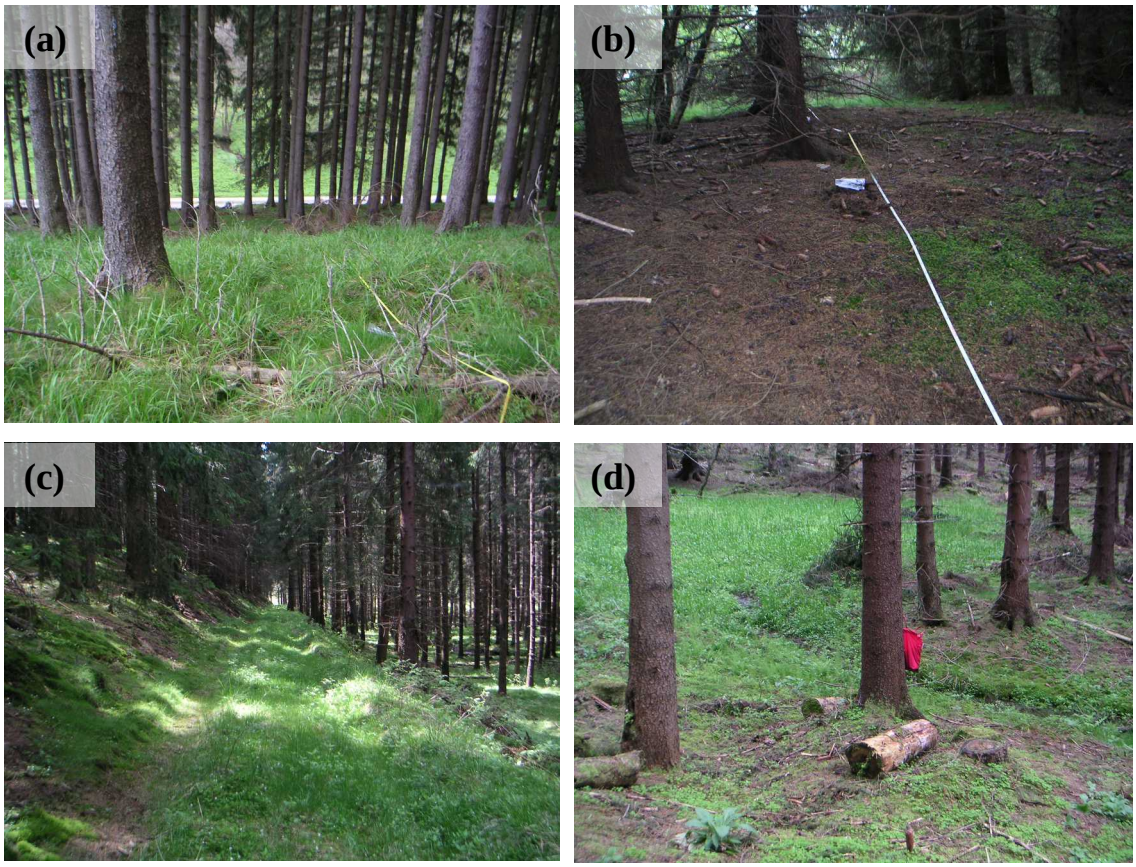

Fig. 4.2. Pictures of landscape of Picea abies forest in Harz Mts. (a): site A; (b): site B; (c): site C; (d): site D.

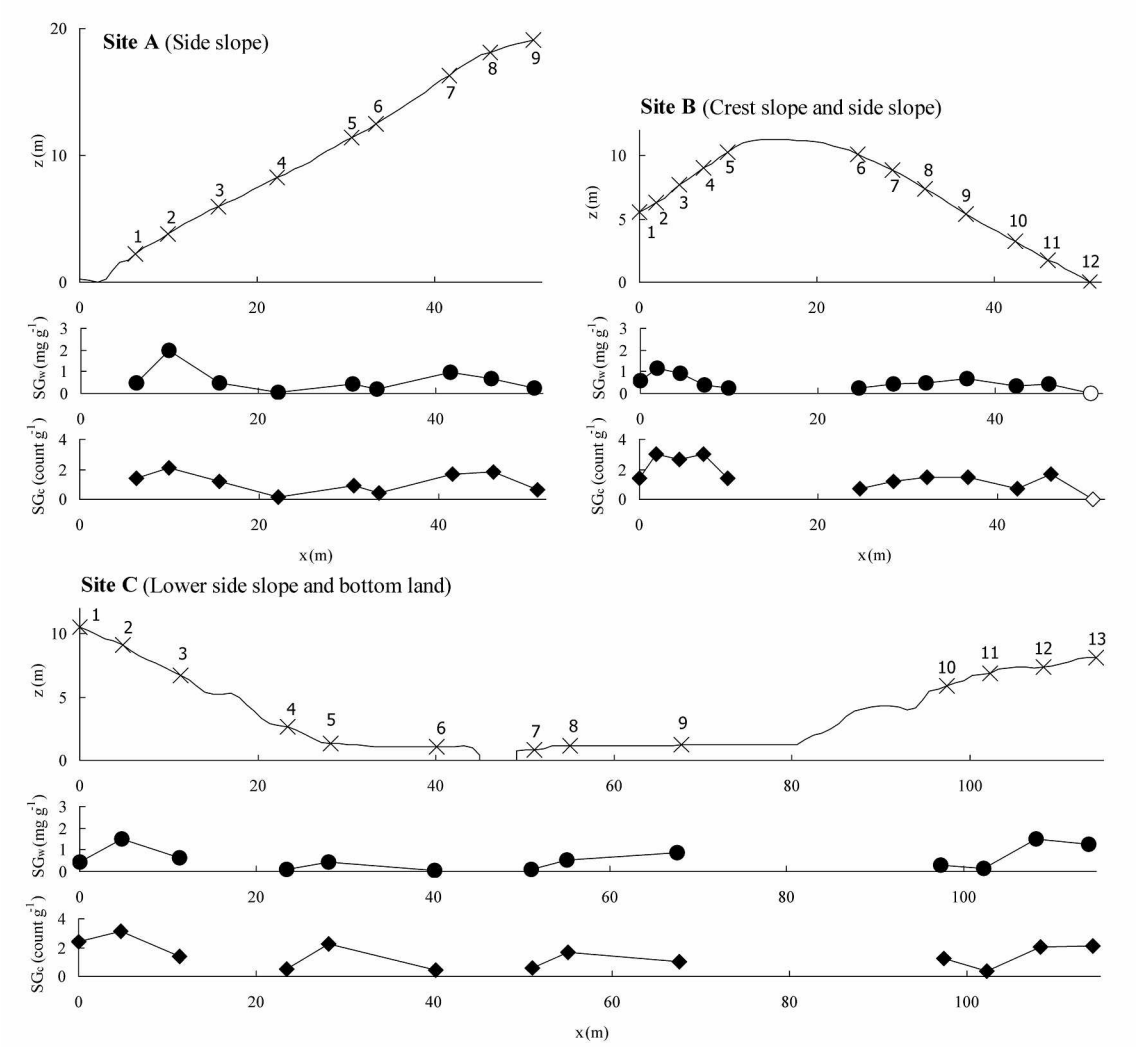

Fig. 4.3(1). Micro-topography of site A~C and the distribution of sclerotial biomass. Sclerotial biomass was defined by weight (SG_w) and count (SG_c) of air-dried sclerotia per air-dried soil. First graph for each site is micro-topography, and × is sampling point. Second graph is SG_w (mg g^{-1}) (●). Third graph is SG_c (count g^{-1}) (♦). White circle (○) and diamond (◊) in lower two graphs means no sclerotia was collected from soil.

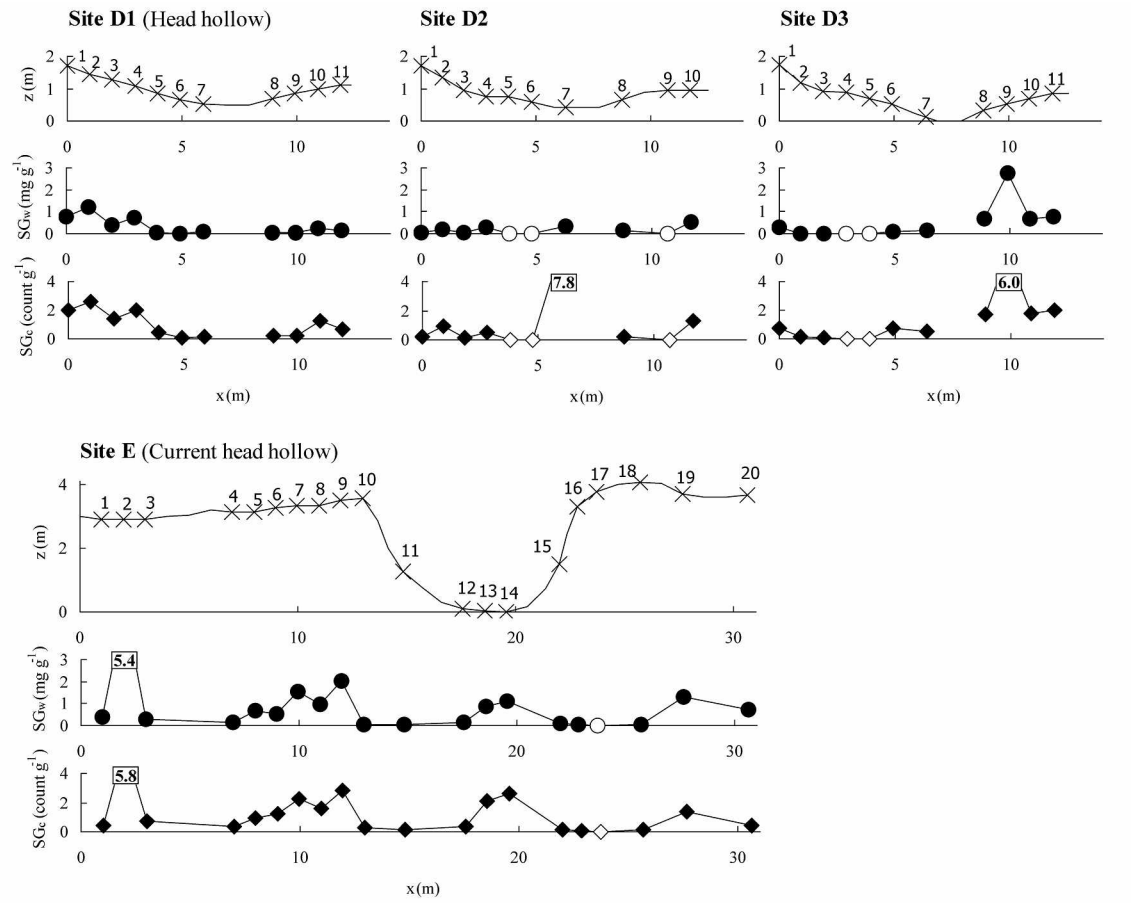

Fig. 4.3(2). Micro-topography of site D, E and the distribution of sclerotial biomass.

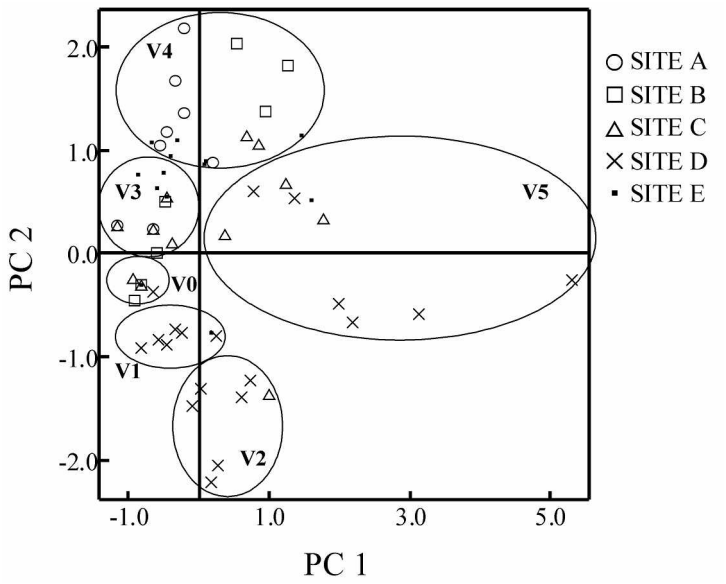

Fig. 4.4. Six types (V0 ~ V5) of vegetation categorized from the result of PCA of floor vegetation.

Fig. 4.5. Representative pictures of six types (V0 ~ V5) of vegetation. Floor vegetation is scarce in V0 and V3, and is plenty in V2, V4 and V5. V5 is characterized by its rich diversity on floor vegetation.

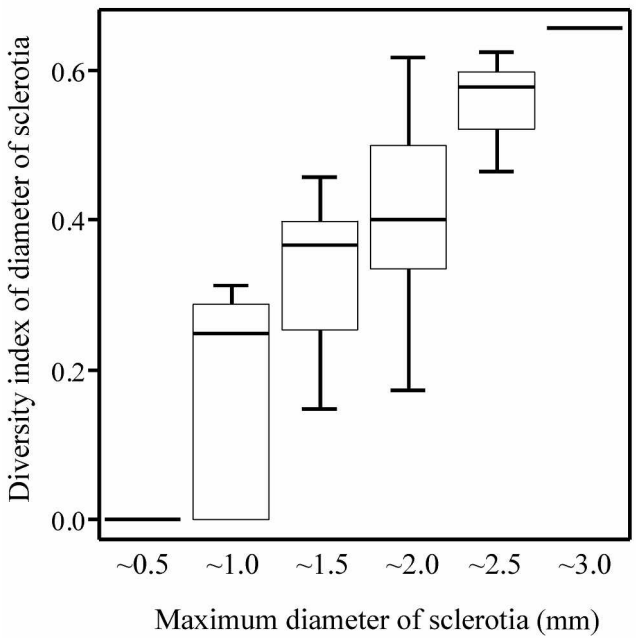

Fig. 4.6. Shannon-Weiner diversity index calculated for the size class (each 0.5 mm in diameter) of sclerotia.

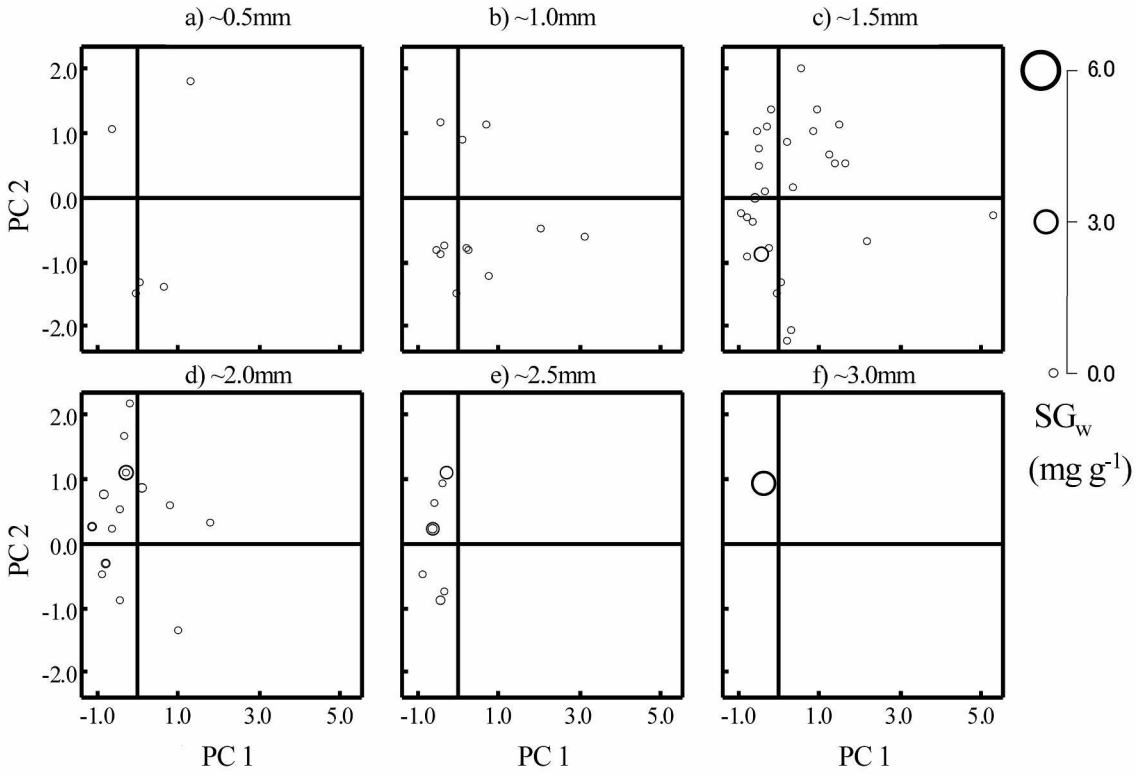

Fig. 4.7. Distribution of sclerotia described by magnitude of sclerotial biomass by weight (SG_w) for each class of maximum diameter of sclerotia in concern with floor vegetation (PC 1 and 2).

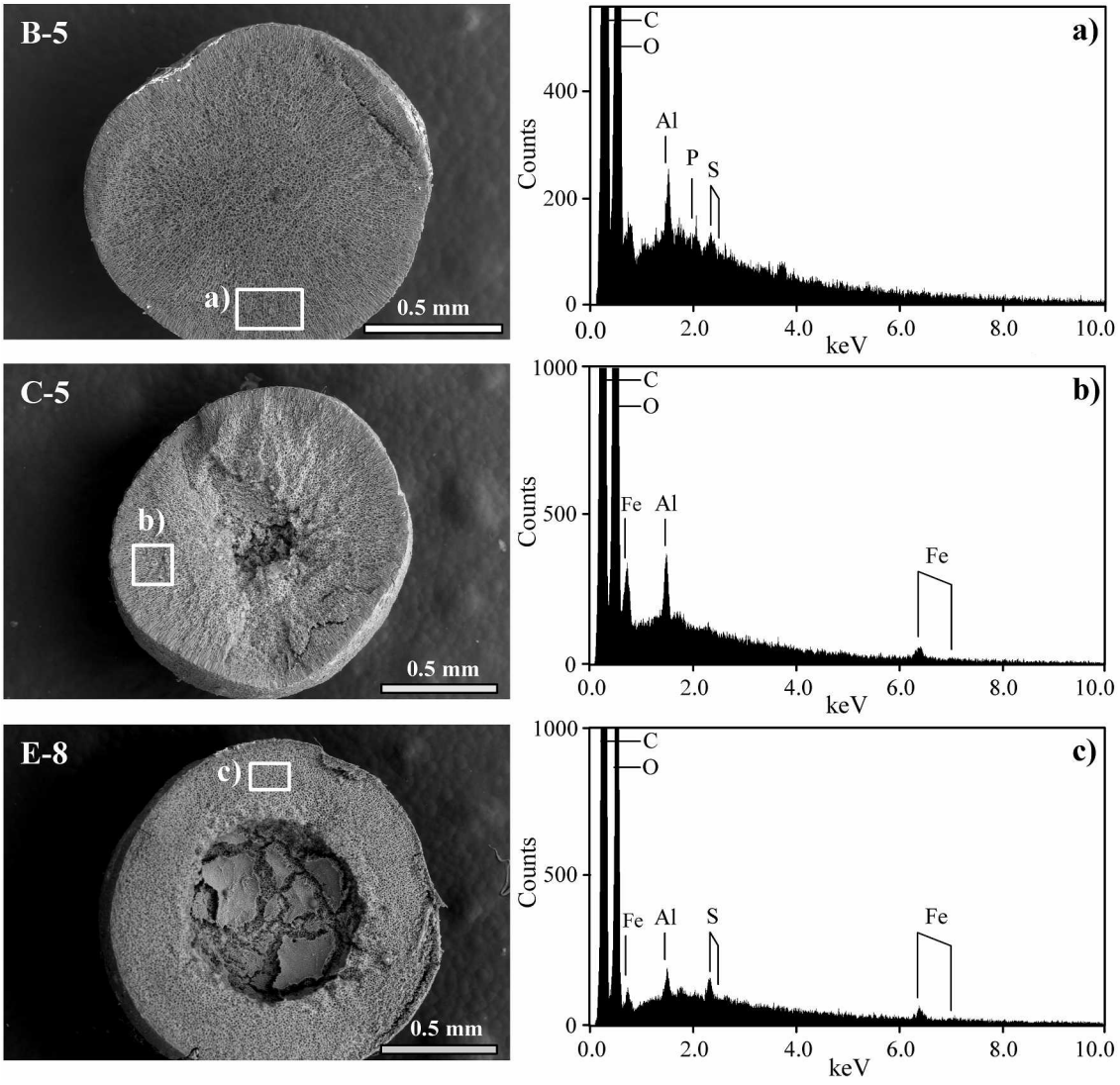

Fig. 4.8. The morphological features observed by SEM and spectra by EDS analysis of three sclerotia from points B-5, C-5, E-8. Chemical compositions were qualitatively observed for the open squared area (a~c).

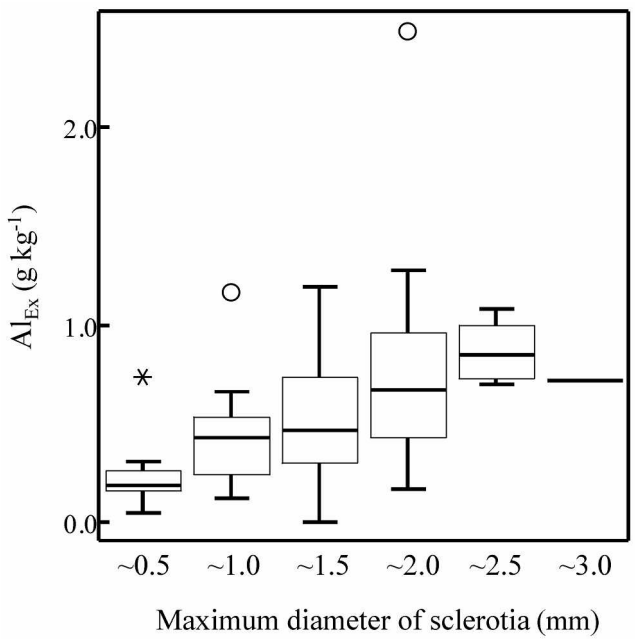

Fig. 4.9. Relationship between contents of Al_{Ex} (g kg^{-1}) and the maximum diameter of sclerotia. White circle (○) and asterisk (*) indicate outlier and extreme value, respectively

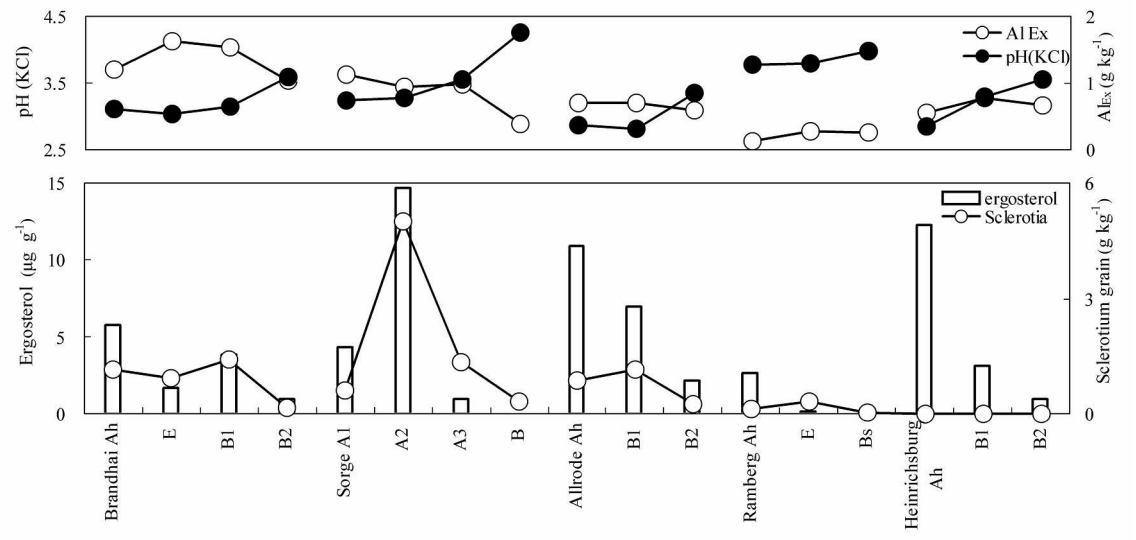

Fig. 4.10. Relationship between content of ergosterol in soil and sclerotial biomass in the ergosterol -rich soil profiles of Brandhai, Sorge, and Allrode in Harz Mts. Correlation was not found in the ergosterol-rich Heinrichsburg soil profile and the content of Al_{Ex} was relatively low despite of low pH (revised from Watanabe *et al*., 2004b).

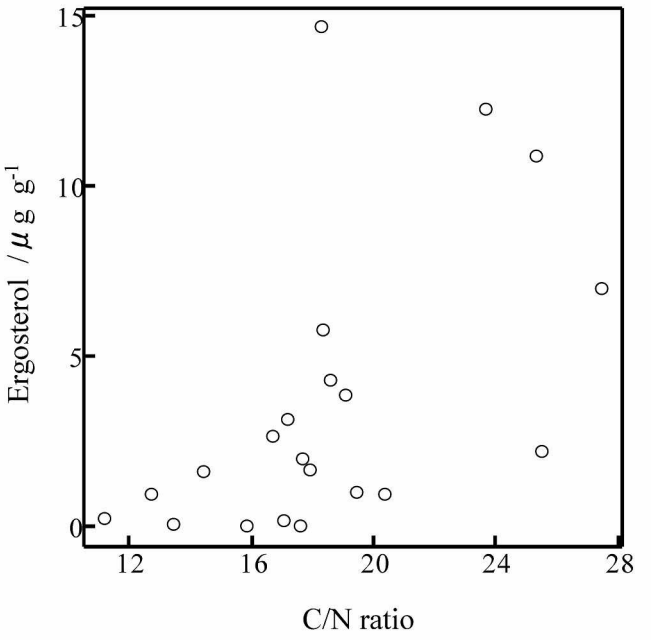

Fig. 4.11. C/N ratio increased in soils with high content of ergosterol (data are taken from Watanabe *et al.*, 2004b).

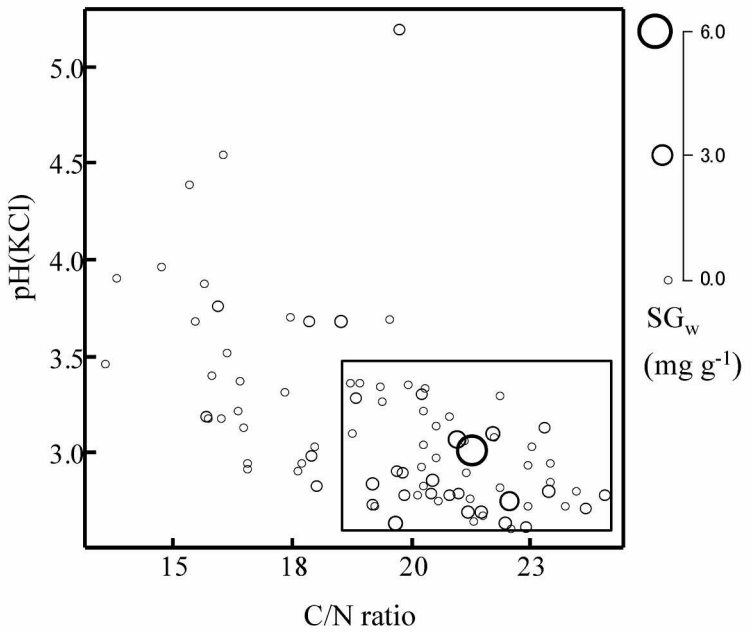

Fig. 4.12. Distribution of sclerotial biomass by weight (SG_w) in relation to pH (KCl) and C/N ratio.

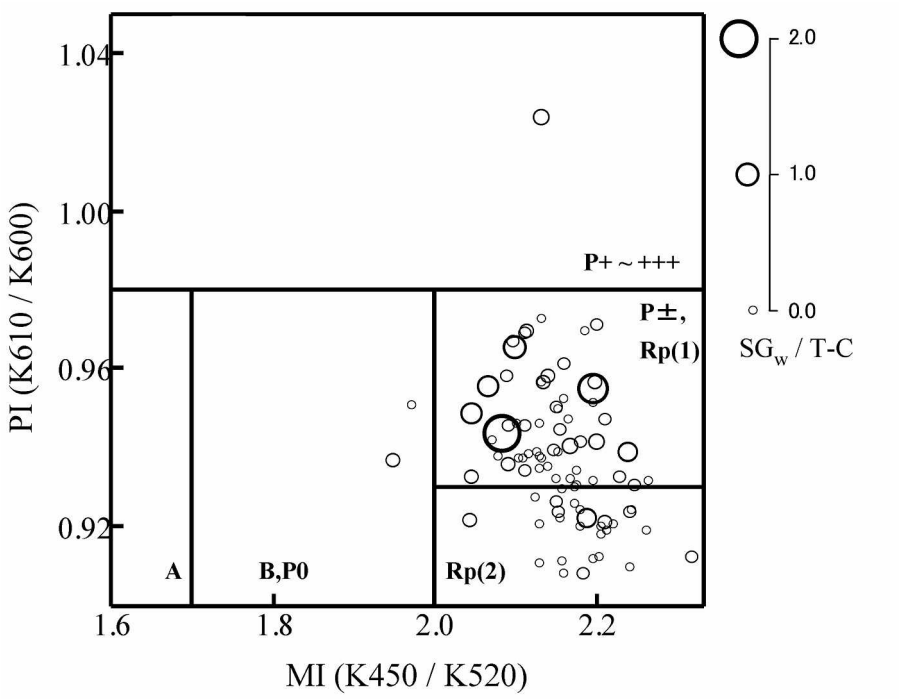

Fig. 4.13. Distribution of SG_w/T-C ratio in relation to MI and PI. As it is known that approximately 50 wt% of sclerotium consists of carbon, contribution of sclerotial carbon to T-C can be estimated from SG_w/T-C ratio.

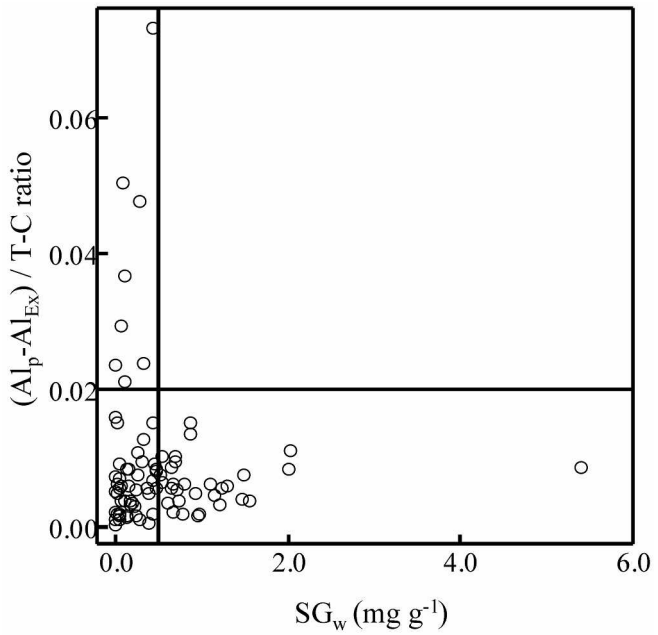

Fig. 4.14. Relationship between Al_p (Al_p−Al_{Ex})/T-C and SG_w.

Chapter 5

Variance and range of geographical distribution of sclerotia

5.1 Introduction

C. geophilum is known for its extremely wide habitat range. Ectomycorrhiza of *Cenococcum* species have been observed above the Arctic Circle in Alaska and Canadian High Arctic (75°33'N, 84°40'W) and as an important symbiont of trees at timber line in the Washington and Oregon Cascade mountain range (Haselwandter and Read 1982; Trappe 1988; Bledsoe *et al*. 1989). In addition, Vogt *et al*. (1981) estimated the biomass of sclerotia of *C. geophilum* in a 23- and 180-year-old subalpine fir (*Abies amabilis*) stands which are characterized by a cool climate to be 2300 kg ha^{-1} $year^{-1}$ and 3000 kg ha^{-1} $year^{-1}$, respectively, and reported maximum distribution in autumn season in young stands.

In this chapter, seasonal change of distribution of sclerotia, and distribution along an altitudinal gradient in temperate and arctic-alpine climatic zones, are demonstrated in central and northern Japan to discuss the variance and range of sclerotia in field.

5.2 Materials and methods

Surface soils (approximately 0~5 cm in depth) of Mt. Mito (Tokyo) and Lake Tazawa plateau (Akita pref.) were investigated to clarify the seasonal change of distribution of the sclerotia. Surface soils of Mt. Iwaki (Aomori pref.) and Mt. Ontake (Gifu pref.) were investigated to clarify the distribution of the sclerotia along an altitudinal gradient in temperate and arctic-alpine climatic zones.

At Mt. Mito, to examine seasonal change of distribution of sclerotia, samples were collected every month (from April to November 2007). Two site (site A: 35°44'12"N, 139°00'46"E, 1480 m; site B: 35°44'28"N, 139°01'29"E, 1250 m) were selected and three samples were collected monthly from approximately 2×2 m area of slope in *Fagus* forest (Fig. 5.1).

At Lake Tazawa plateau, soils were collected with micro-topographical investigation on *Fagus* forest slope (39°47'52"N, 140°46'59"E; Fig. 5.2). Field investigations were carried out in Nov. 2006, May 2007 and Nov. 2007. Angle of every 1 m was measured along a line of approximately 160 m, and surface 18 soil samples were collected at every 10 m along the measurement line.

At Mt. Iwaki (Iw), surface soils were taken from 22 points along two gradient: one along the driveway (point 1~10; 40°37'41"N, 140°15'15"E ~ 40°39'14"N, 140°18'02"E); and another along the trail (point 11~22; 40°37'56"N, 140°16'04"E ~ 40°39'07"N, 140°17'26"E) as shown in Fig. 5.4. Field investigations at Mt. Iwaki were carried out on July and September 2006. Pediment area of Mt. Iwaki is covered with secondary forest (mainly *Quercus serrata*). Point 4~7 and 14~21 is *Fagus* forest, and higher region beyond the forest line are covered with *Betula ermanii, Alnus maximowiczii* or *Sasa kurilensis.*

At Mt. Ontake (On), surface soils were taken from 16 points (35°55'29"N, 137°26'41"E ~ 35°54'37"N, 137°28'59"E) along trail as shown in Fig. 5.5. Field investigation at Mt. Ontake was carried out on June 2006. *Abies veitchii* and *Abies mariesii* are the main vegetation at point 1~11. The forest line is around point 12, and higher regions are covered with

Pinus pumila.

Sclerotia were carefully picked up from air-dried and homogenized surface soil samples using tweezers and kept under air-dried condition. 10~30 g of air-dried soil were stirred after adding 300 ml of distilled water to collect all sclerotia from the float of the suspension. The counts of sclerotia from Mt. Iwaki and Mt. Ontake were obtained by following 7 divisions of their diameter range (0.2~0.5, 0.5~1.0, 1.0~1.5, 1.5~2.0, 2.0~2.5, 2.5~3.0, and 3.0~3.5 mm). Sclerotial biomass was defined by weight (SG_w) and count (SG_c) of air-dried sclerotia per air-dried soil. Using soils from Mt. Iwaki and Mt. Ontake, pH(KCl) analysis were carried out on air-dried soil samples passing through a 2 mm sieve.

Air-dried sclerotia were sliced into half pieces using a sterilized knife to observe the morphological features and chemical composition of transverse wall. For this operation, grains were selected from each 9 points of both Mt. Iwaki and Mt. Ontake (point 3, 5, 6, 8, 11, 14, 18, 20 of Mt. Iwaki and point 1, 3, 4, 6, 8, 9, 11, 12, 15 of Mt. Ontake). Chemical composition was obtained for these samples using a scanning electron microscope (SEM) and energy dispersion X-ray spectrometer (EDS) (JSM-6490LA, JEOL Ltd, Tokyo, accelerating voltage of 15 eV).

5.3 Seasonal change of distribution of sclerotia

5.3.1 Mt. Mito

Fig. 5.5 shows the monthly change of SG_c in Mt. Mito and daily mean temperature and precipitation observed in Ogochi dam, near Mt. Mito (climatic data were taken from website of Japan Meteorological Agency). SG_c ranged 3.6~5.5 count g^{-1} in site A, and 1.5~3.9 count g^{-1} in site B. Average of SG_c throughout 8 months in site A and B were 4.7 and 2.1 count g^{-1}, respectively. In April, SG_c in site A was 5.5 count g^{-1} and gradually decreased with increase of

temperature to 3.7 count g^{-1} in June. This decrease can be understood as decomposition of sclerotia by activity of micro-organisms, supposedly including germination of fungal mycelia. After stable 3 months, SG_c increased rapidly to 5.4 count g^{-1} in September, before decline of temperature. From this fact, aim of formation of sclerotia for this fungus can be considered as provision for winter season. Similarly to site A, SG_c of site B decreased from 3.4 to 1.5 count g^{-1} from April to May. But unlikely to site A, no increase of SG_c was observed in autumn season in site B. These facts suggest that formation of sclerotia does not occur homogenously in surface forest soils.

5.3.2 Lake Tazawa plateau

Measurement lines of three season in *Fagus* forest at Lake Tazawa plateau are shown in Fig. 5.6. Fig. 5.7 describes SG_c along measurement lines. Average of SG_c for three seasons were 2.7, 2.3, 3.9 count g^{-1}, respectively. At steep side slope, drastic changes of SG_c were observed throughout three seasons and a slight downward transportation of sclerotia could be recognized. Such steep location may have vigorous material circulation, or soil erosion, and it will be difficult for *C. geophilum* to forecast environmental changes. Formation of large amount of sclerotia could be considered as diffusion with soil erosion in steep slope. At crest slope, oscillation of relatively stabilized distribution of sclerotia was observed.

5.4 Distribution of sclerotia along an altitudinal gradient

Results of SG_w, SG_c, pH(KCl) of soils from Mt. Iwaki and Mt. Ontake are summarized in Table 5.1 and 5.2, respectively. At Mt. Iwaki, the maximum sclerotial biomass was observed in *Fagus* forest: 2.3 g kg^{-1} by SG_w (point 5) and 23.5 count g^{-1} by SG_c (point 6). At Mt. Ontake,

point 1 had the maximum biomass: 2.8 g kg^{-1} by SG_w and 4.4 count g^{-1} by SG_c. The distribution of sclerotia can be interpreted by Warmth Index (WI). WI was calculated as follows according to Kira (1976):

$$WI = \sum (t - 5) \qquad (5.1$$

where t is the monthly average temperature which is over 5 ºC. Average monthly temperatures were taken from Mesh Climatic Data 2000 (Japan Meteorological Agency, 2002). Fig. 5.8 shows the relationship between WI and SG_c. Abundant formations of sclerotia were distributed in its suitable range: approximately $35 < WI < 60$ and a peak around $WI = 45$. On the contrary, pH(KCl) values were low in this area (Fig. 5.9). According to Nogami (1994), subalpine and cool-temperate forest vegetation zones can be recognized as $23 < WI < 45$ and $45 < WI < 74$, respectively. Sclerotia were distributed in these vegetation zones with clear peak at boundary of subalpine and cool-temperate zone. But clear correlation with actual vegetation could not be recognized. Considering that the sclerotia potentially persist thousands of years, such distribution of sclerotia, being not strongly influenced by vegetation is likely to be cumulated evidence of success to preserve their species for long periods.

Fig. 5.10 and 5.11 shows SG_c, which was classified by diameter range of sclerotia at Mt. Iwaki and Mt. Ontake. In case of Mt. Iwaki, small sclerotia (<0.5 mm in diameter) tended to be formed abundantly in higher altitudes. This fact can be suggested as strategy of diffusion by *C. geophilum*. On the contrary, relatively large sclerotia were observed in pediment area even though the amount of sclerotia was low. This can be understood as preservation of sclerotia. In case of Mt. Ontake, such tendency was not recognized. But as shown in Fig. 5.12, large amount of non-spherical sclerotia with few amount of spherical sclerotia were collected from point 15. Although little information has emerged on these non-spherical sclerotia, one possible hypothesis is “rapid formation” of sclerotia. In such adverse condition, *C. geophilum* have a

possibility to form sclerotia rapidly and non-spherically. These sclerotia might be an important source of assimilated carbon from host species, for micro-organisms in subalpine forest soils. Experiments for maturation of sclerotia in agar medium in extreme conditions will provide supplementary information in this hypothesis.

Table 5.3 shows the result of EDS analysis. Fe/Al ratios of internal and wall part of sclerotia had high value comparing to sclerotia studied in chapter 3. Fe/Al ratio was gradually improved to higher region. Point 6 at Mt. Iwaki, which contain large amount of sclerotia, showed relatively high value of Fe/Al ratio. Point 6, 8, 9 of Mt. Ontake showed idiosyncratic low value of Fe/Al ratio. Essén *et al*. (2006) reported occurrence of siderophore in podzolic soil. High Fe/Al ratio in high altitudes may be able to be explained by siderophore of *C. geophilum*. In addition, absorption of Al and Fe may controlled by fungal potential to produce sclerotia and soil chemical environment. Quantitative analysis on Al and Fe in sclerotia will serve for clarifying these subjects.

5.5 Conclusion

Distribution of sclerotia showed fluctuational variance, and aim of formation of sclerotia for this fungus were considered as provision for winter season. The decrease of sclerotia could be understood as decomposition of sclerotia by activity of micro-organisms, supposedly including germination of fungal mycelia.

Sclerotia had a suitable range of distribution in subalpine and cool-temperate vegetation zones with clear peak at boundary of these two zones. Distribution of sclerotia, being not strongly influenced by vegetation is likely to be cumulated evidence of success to preserve their species for long periods. As strategy of *C. geophilum*, formation of small sclerotia, and supposedly rapid formation of non-spherical sclerotia in higher altitudes were suggested as

diffusion of species. These sclerotia might be an important source of assimilated carbon from host species, for micro-organisms in subalpine forest soils. On the contrary, formation of relatively large sclerotia observed in pediment area was suggested as preservation of sclerotia. In addition, absorption of Al and Fe may controlled by fungal potential to produce sclerotia and soil chemical environment.

Table 5.1. Results of SG_w, SG_c and pH(KCl) of soils from Mt. Iwaki.

Point (Mt. Iwaki)	Elevation m	pH(KCl)	SG_w mg g^{-1}	SG_c count g^{-1}
1	400	5.07	0.0	0.7
2	460	3.89	0.1	0.7
3	595	3.28	0.4	1.7
4	640	2.64	0.8	1.4
5	710	2.96	2.3	3.4
6	790	3.39	1.8	23.5
7	890	3.14	0.4	4.4
8	1040	3.77	0.6	6.6
9	1364	3.09	0.1	0.4
10	1480	4.16	0	0
11	484	3.56	0.4	2.8
12	595	3.2	0.6	6.0
13	677	3.31	1.2	5.0
14	698	3.53	0.3	3.2
15	794	3.21	0.7	5.5
16	834	3.52	0.2	2.8
17	853	2.9	0.6	11.2
18	928	3.09	0.9	2.0
19	957	2.76	1.3	3.2
20	1037	2.89	2.2	2.3
21	1131	3.53	0.9	16.3
22	1156	3.65	0.4	4.6

Table 5.2. Results of SG_w, SG_c, pH(KCl) of soils from Mt. Ontake.

Point (Mt. Ontake)	Elevation m	pH(KCl)	SG_w mg g^{-1}	SG_c count g^{-1}
1	1715	3.35	2.8	4.4
2	1740	2.78	0.4	0.8
3	1870	3.82	0.5	3.2
4	1910	2.7	0.5	2.7
5	1940	2.64	0.1	0.1
6	2020	2.88	0.5	1.5
7	2030	2.79	0.4	1.3
8	2100	2.84	2.1	2.7
9	2200	3.07	0.7	2.9
10	2320	2.82	0.8	0.9
11	2460	3.57	0.2	1.5
12	2530	3.94	0.4	2.3
13	2600	3.47	0.4	0.7
14	2645	3.18	0.6	1.0
15	2740	4.1	1.5	2.9
16	2770	3.5	0.2	0.5

Table 5.3. Result of EDS analysis on internal and wall part of sclerotia.

Point	Fe/Al (wt ratio)	
	Internal	Wall
(Mt. Iwaki)		
3	1.6	0.4
5	1.0	0.9
6	1.4	3.6
8	0.6	1.4
11	0.1	0
14	0.5	0.3
18	1.8	0.7
20	5.5	0.6
22	14.8	13.1
(Mt. Ontake)		
1	2.6	1.5
3	4.3	3.8
4	65.2	6.7
6	2.8	2.3
8	2.0	1.3
9	0	0
11	127.9	21.1
12	30.6	7.1
15	12.6	14.5

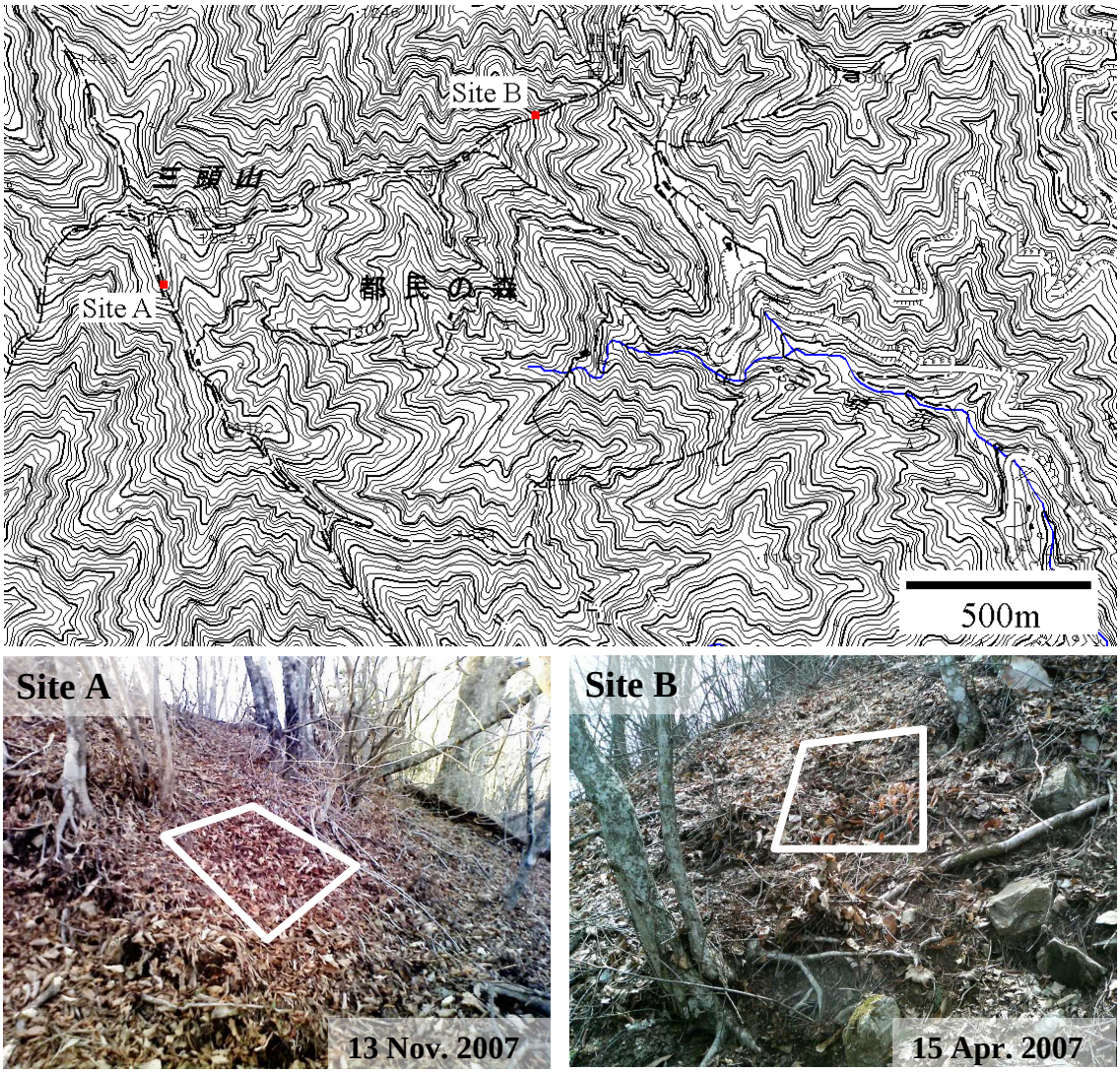

Fig. 5.1. Topographic map and pictures of landscapes of study area at Mt. Mito. Surface soil samples were collected in open squared area of each site A and B.

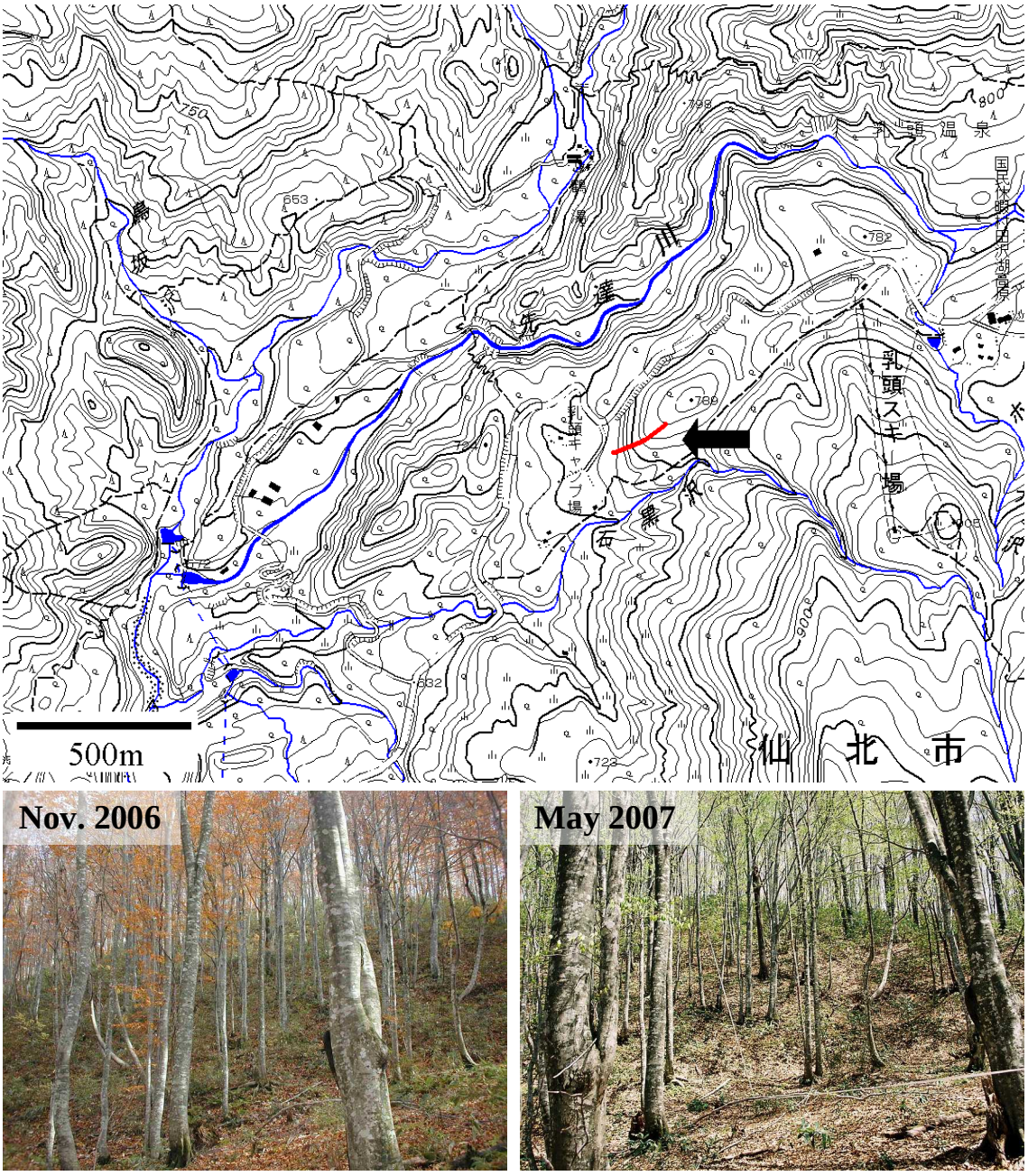

Fig. 5.2. Topographic map and picture of landscapes of *Fagus* forest slope at Lake Tazawa plateau, on Nov. 2006 (a) and May 2007 (b). Red line in topographic map indicates measurement line.

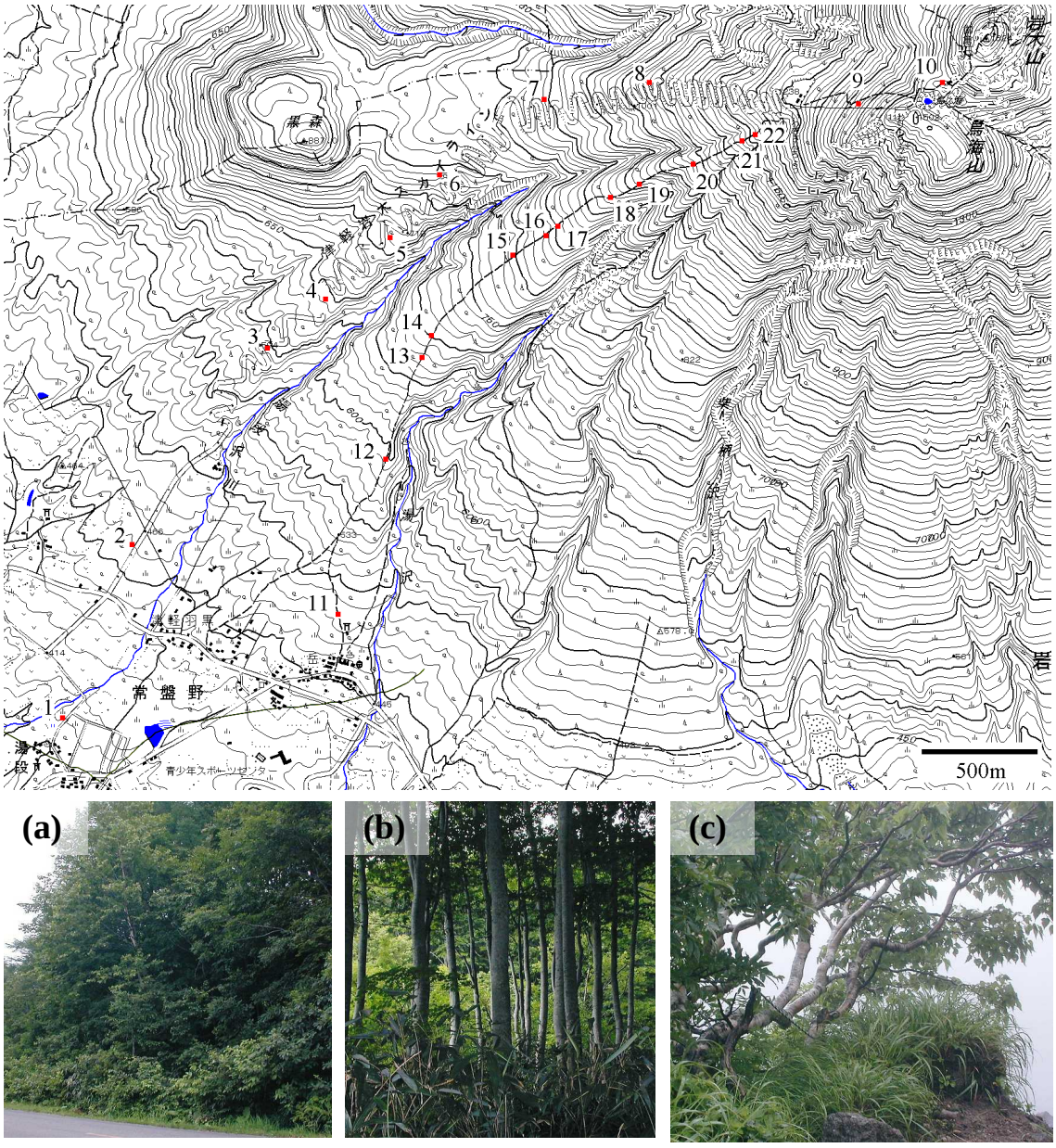

Fig. 5.3. Topographic map and picture of landscapes of study area at Mt. Iwaki. Numbers 1~22 in topographic map indicate points of surface soil sampling. Picture (a) is the secondary forest near point 2, (b) is the *Fagus* forest near point 6, and (c) is the *Betula ermanii* forest near point 9, over the forest line.

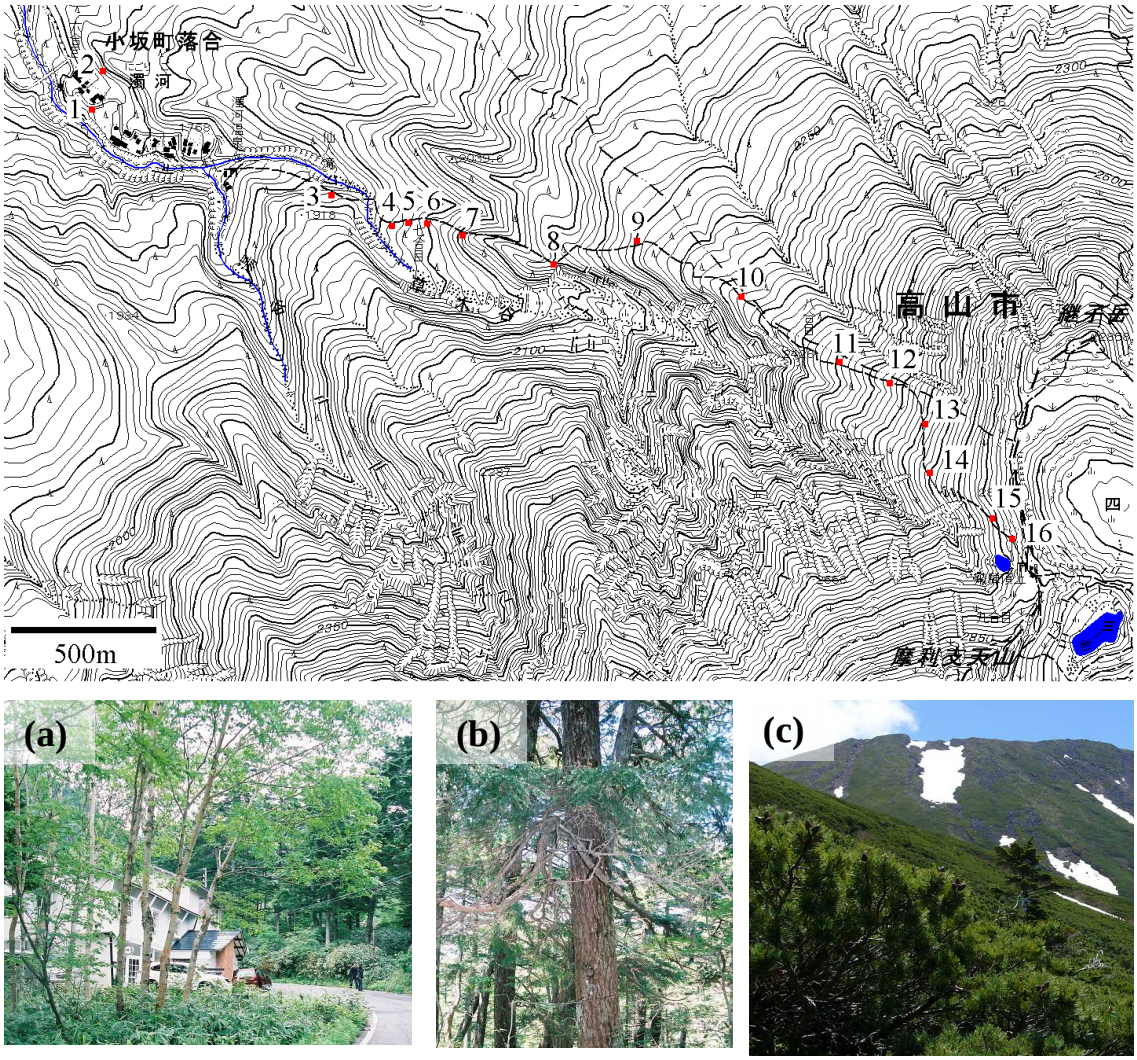

Fig. 5.4. Topographic map and picture of landscapes of study area at Mt. Ontake. Numbers 1~16 in topographic map indicate points of surface soil sampling. Picture (a) is the *Betula ermanii* forest near point 1, (b) is the *Abies veitchii* and *Abies mariesii* forest near point 8, and (c) is the *Pinus pumila* forest near point 14, over the forest line.

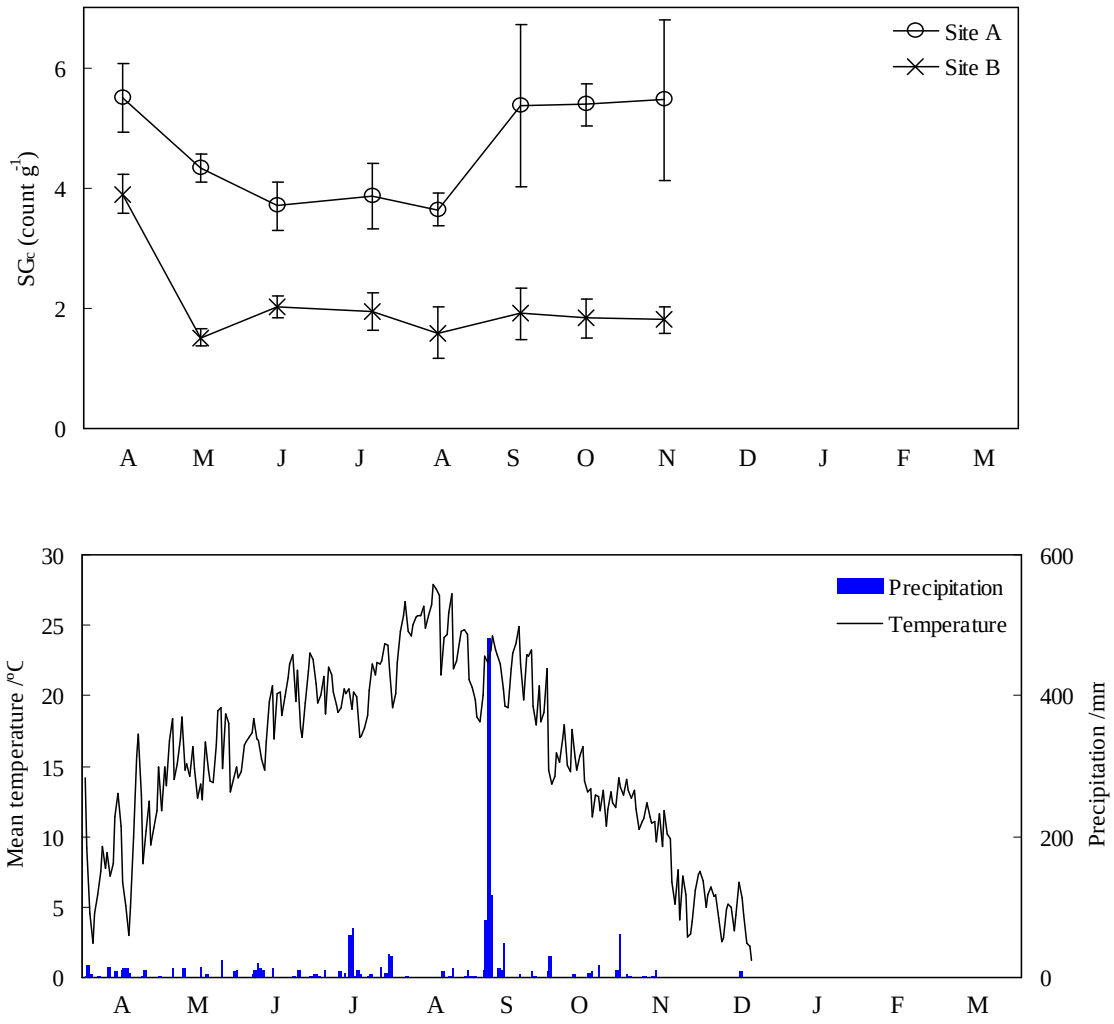

Fig. 5.5. Seasonal change of SG_c in Mt. Mito and daily mean temperature and precipitation observed in Ogochi dam, near Mt. Mito (climatic data were taken from website of Japan Meteorological Agency).

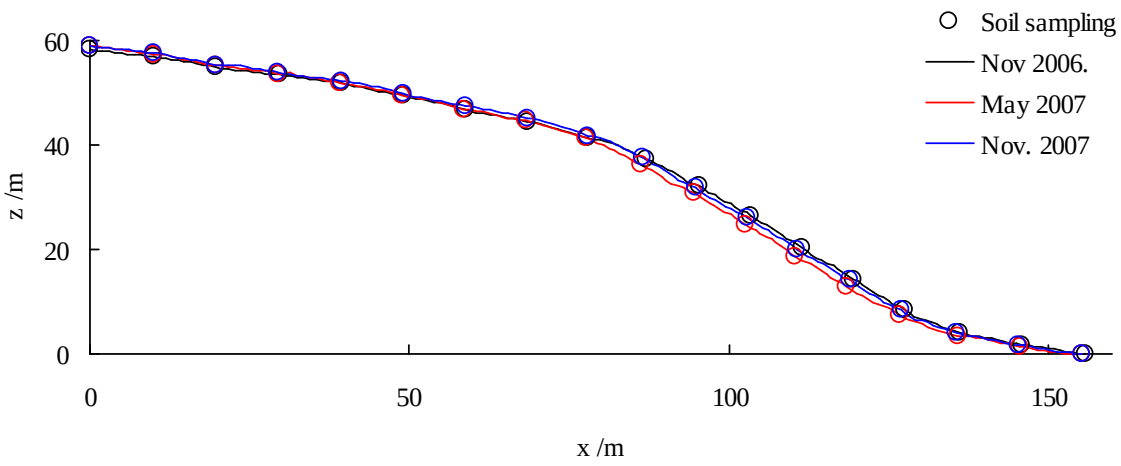

Fig. 5.6. Three measurement lines of *Fagus* forest in Lake Tazawa plateau.

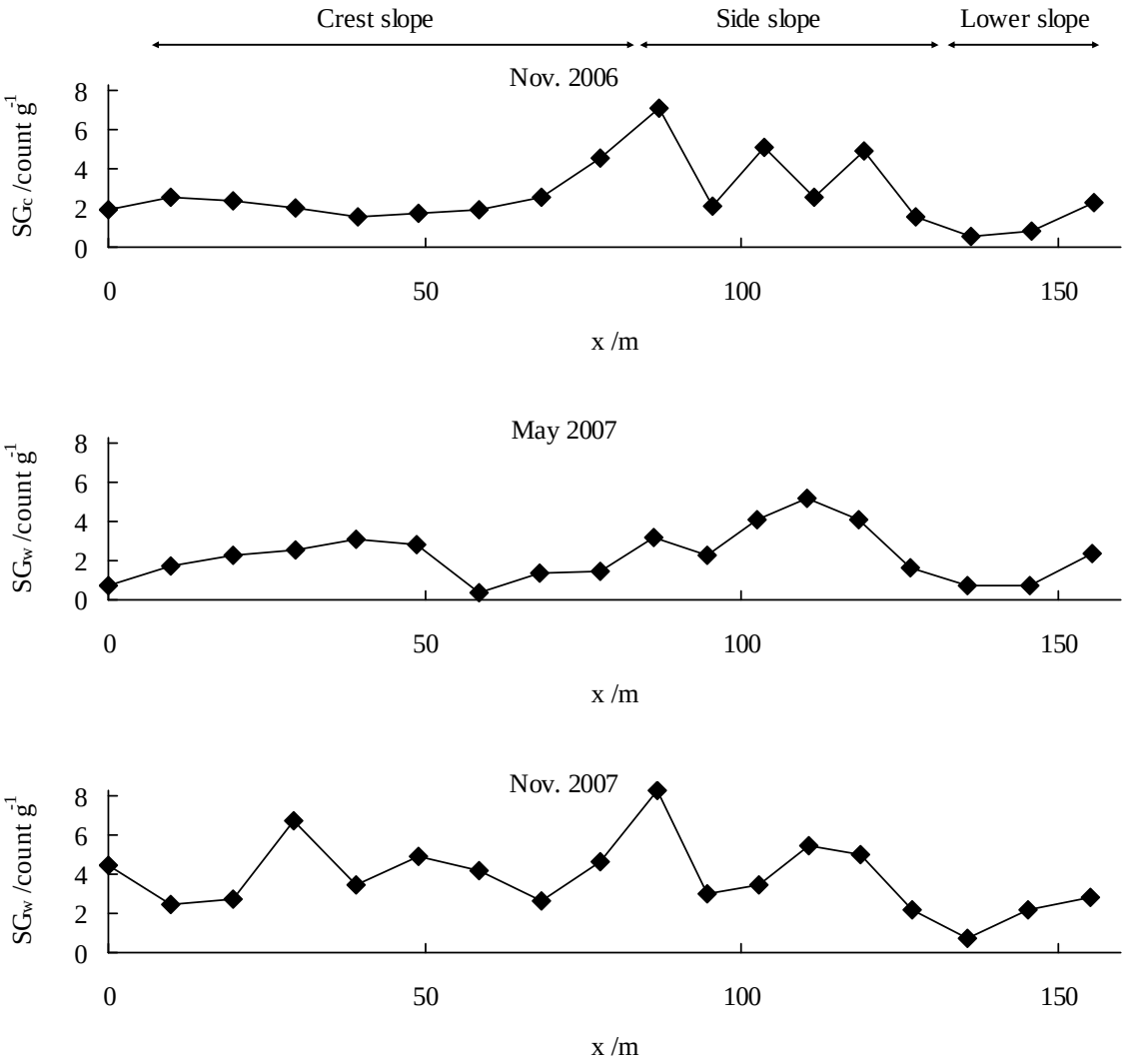

Fig. 5.7. Results of SG_c for surface soil along measurement lines.

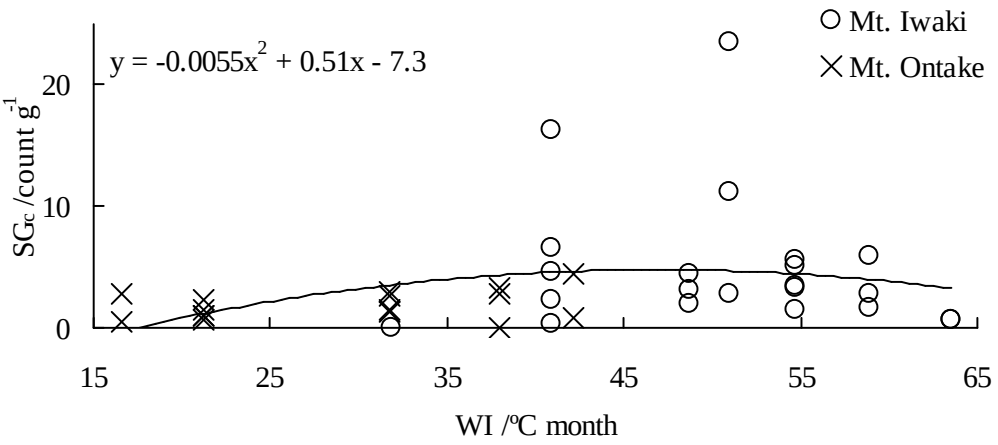

Fig. 5.8. Relationship between Warmth Index (WI) and SG_c.

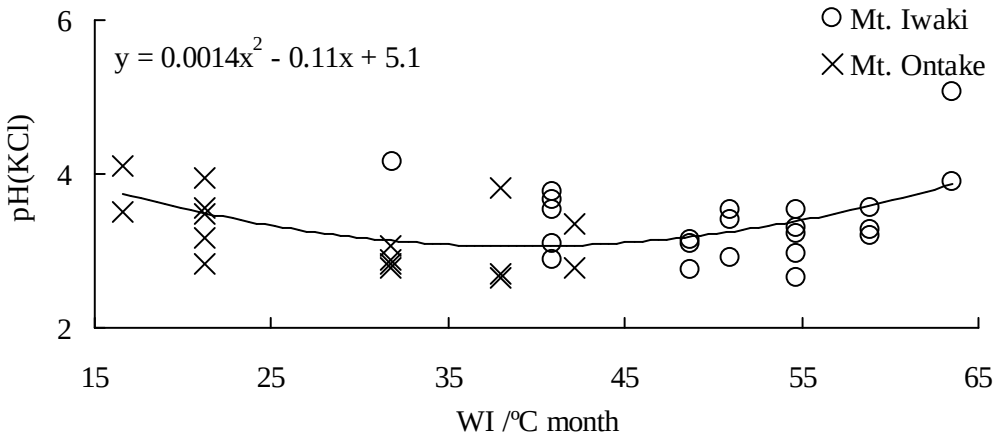

Fig. 5.9. Relationship between WI and pH(KCl).

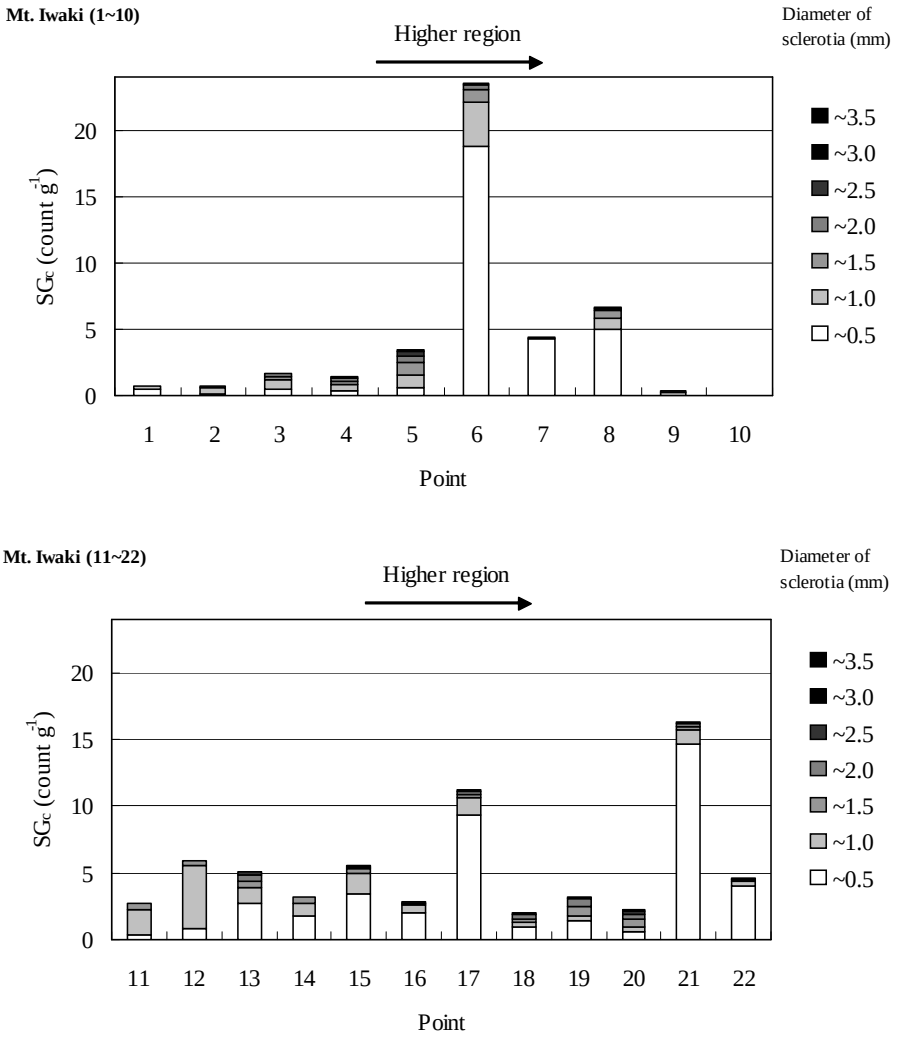

Fig. 5.10. SG_c classified by diameter range of sclerotia at Mt. Iwaki.

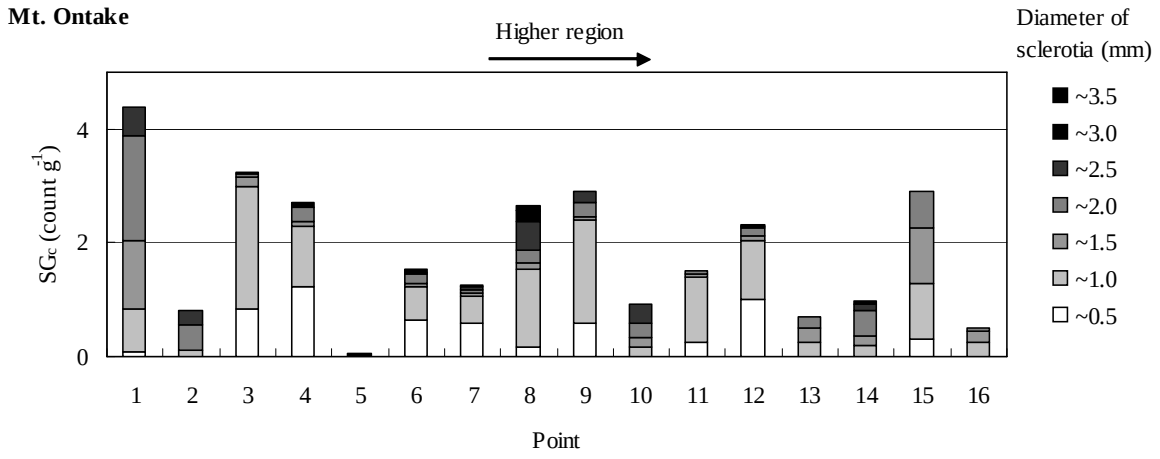

Fig. 5.11. SG_c classified by diameter range of sclerotia at Mt. Ontake.

Fig. 5.12. Picture of a) spherical sclerotia collected from point 2 (1740 m) and b), c) non-spherical sclerotia collected from point 15 (2740 m) at Mt. Ontake.

Chapter 6

Conclusion

6.1 Concluded remarks

In this study, chemical and physical characteristics, and distributional properties of sclerotia of *C. geophilum* and related species were examined to obtain deeper knowledge on the nature of sclerotia, the exiting structural organic component in soil. Distributional properties of sclerotia and its role as a soil organic component were summarized as follows.

The mean concentrations of major elements in sclerotia from Mt. Myoko were C (47.6 %), O (30.2 %), H (3.3 %), Al (1.4 %), N (0.78 %). Carbon in sclerotia was in the form of large amounts of *O*-alkyl C, which showed strongly and characteristically biological origin, and completely different spectral features to humic and fulvic acids from an allophanic Andosol. On the contrary, low content of N, P, and S suggested that sclerotia do not exist as mycelia, but as an abiotic organic component. Sclerotia were suggested as transitive component from fungal metabolites to humic acid, en route toward humic acids from “cellulose and other non-lignin substrates”. The chemical composition of sclerotia was strongly influenced by soil-environmental condition. The absorption, or retention of Al and Fe ions together with C by biosynthetic process may have a role to reduce phyto-toxic Al and Fe in low pH soils. Al_{SG} and Fe_{SG} were defined as Al and Fe in the form of sclerotia. The content of Fe and Al in sclerotia had close relation to the content of active Al and Fe in soils, and chemical composition of sclerotia was suggested to be strongly influenced by status of active Al and Fe in soil

-environment, and the existence of sclerotia were considered to have possible impact to interact with soil chemical properties. Thus, the characteristics of sclerotia were expected to become an indicator of soil chemical properties.

Role of sclerotia as soil organic component were discussed by interpreting distributional properties of sclerotia that are remaining in low pH soils of *Picea abies* forest, Harz Mts. From significant relationships between sclerotial biomass and soil chemical properties, it was clarified that the distribution of sclerotia was regulated by Al_{Ex} content, and was more likely to playing a role towards formation of humus-metal complex in lower pH soils. Absorption of Al and Fe in sclerotia was considered as a Fe reserve for *C. geophilum* itself, and role of retention of phyto-toxic Al in form of biosynthesized humic substances. These results will provide supplementary information in the symbiotic strategies of *C. geophilum* and plants.

Distribution of sclerotia showed seasonal change and aim of formation of sclerotia for this fungus were considered as provision for winter season. The decrease of sclerotia could be understood as decomposition of sclerotia by activity of micro-organisms, supposedly including germination of fungal mycelia. Sclerotia had a suitable range of distribution in subalpine and cool-temperate vegetation zones with clear peak at boundary of these two zones in central and northern Japan. Distribution of sclerotia, being not strongly influenced by vegetation is likely to be cumulated evidence of success to preserve their species for long periods. As strategy of *C. geophilum*, formation of small sclerotia, and supposedly rapid formation of non-spherical sclerotia in higher altitudes were suggested as diffusion of species. On the contrary, formations of relatively large sclerotia observed in pediment area were suggested as preservation of sclerotia. In addition, absorption of Al and Fe may controlled by fungal potential to produce sclerotia and soil chemical environment.

Based on the above mentioned findings, a simple diagram on formation of sclerotia by fungi is illustrated in Fig. 6.1. In biosynthetic process of formation, they are likely to be strongly influenced by their soil-environmental conditions, such as dissolved organic matters,

metal-humus complexes, and minerals. Siderophore will play some role in high Arctic region to extract and utilize Fe from minerals of humus complexes. Fig. 6.2 is a proposal on a dynamic model of formation of sclerotia. Since sclerotia are resting bodies, “live” sclerotia supposedly contain reserve substances. But from our observation, abiotic “dead” sclerotia, luck with N, P persist in soils, and germination from these sclerotia still can be expected. Sclerotia, persisting in soils will chemically and physically decomposed or utilized by micro-organisms through long term and may end up to humic acids. Throughout these processes, sclerotia as soil organic component, play a role of Al retention, Fe reserve, and C pool.

6.2 Further subjects on studying distribution of sclerotia

When we find sclerotia in soil, we may be able to guess low pH and relatively high C/N ratio of soils. If these sclerotia have large diameter, the soil may have high content of Al_{Ex}, or had experienced some event which enriched its content of Al_{Ex} in the past. Formation of sclerotia may be caused by “selfish” requirement of *C. geophilum* to survive or preserve species, but it can be concluded as naturally symbiotically designed phenomenon in soil ecosystems.

Shindo *et al*. (2003) reported large contribution (2.5~33%) of carbon in charred plant fragments to total C in soils which are containing A-type humic acids, and concluded that the charred plant fragments are one of the sources of A-type humic acids in Japanese Andosols. On the other hand, Kumada and Hurst (1967) suggested sclerotia (i.e. micro-organisms residue) as one of the sources of the humic acid “Pg” fraction. Kobayashi *et al*. (2005, 2006) reported chloroform-extractable green fraction (CEGF), whose color is green in alkaline solution, as one of the components of, or a closely related substance to Pg, and considered sclerotia of *C. geophilum* as one of the origins of CEGF. Although the causes of variance of distribution (i.e. decomposition and formation) of sclerotia are not clear, large variance of formation and

decomposition of sclerotia may effect to accumulate P-type humic acids in forest soils. Quantitative analyses on relationships between sclerotia, CEGF, Pg contents in soils from aspect of comparative humus chemistry will support to clarify the implications of sclerotial formation in forest soils.

Cooperative studies with biology, mycology, botany and humus chemistry on formation and decomposition of sclerotia are expected to clarify the ecological dynamics of organic matters in forest soils.

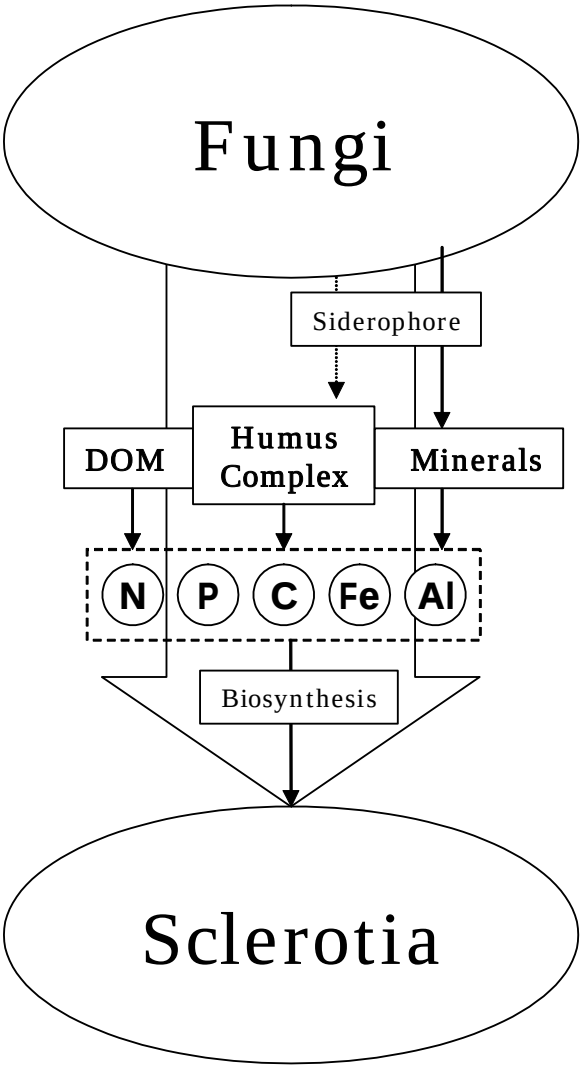

Fig. 6.1. A simple diagram on formation of sclerotia by fungi.

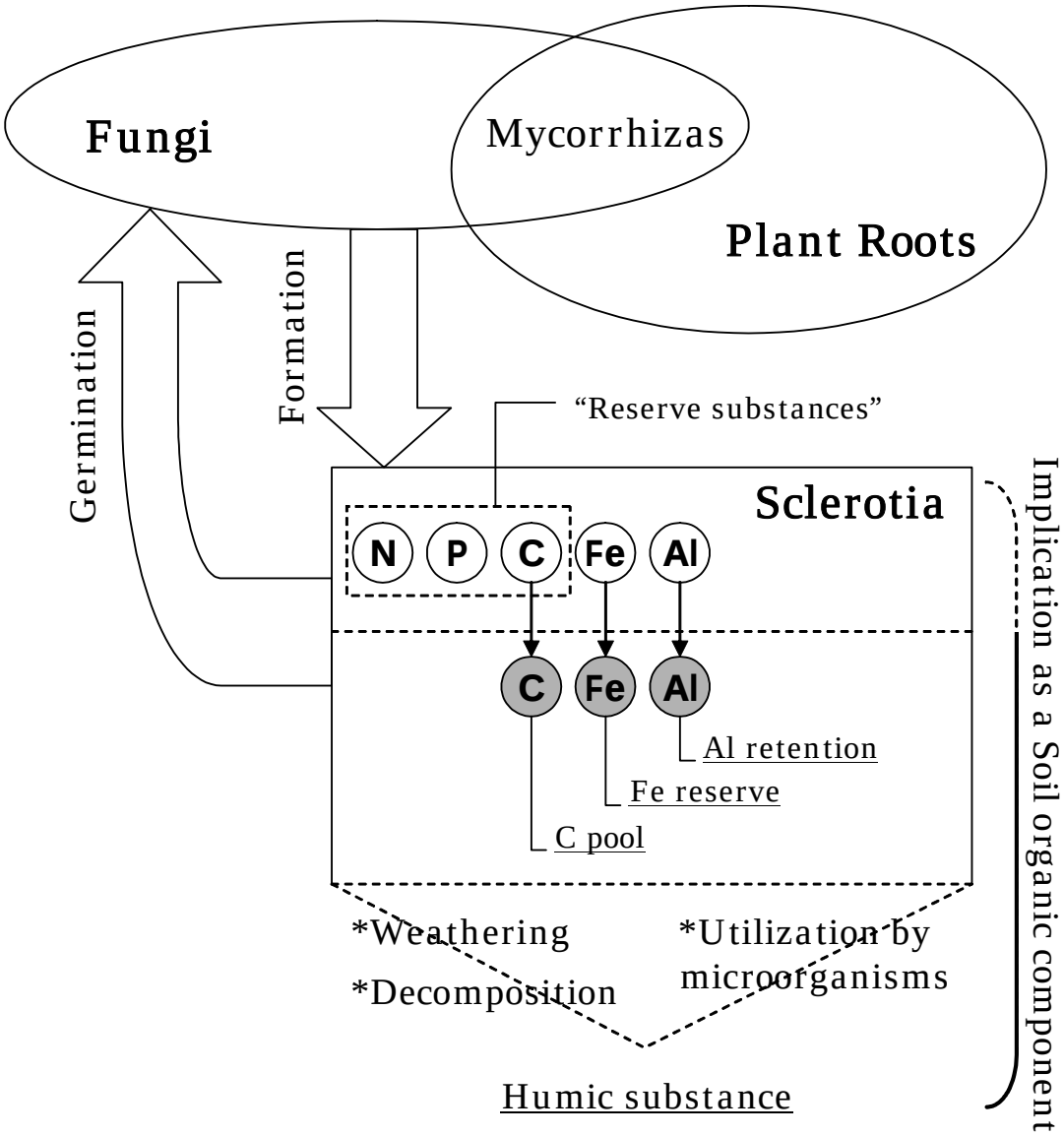

Fig. 6.2. A proposal on a dynamic model of formation of sclerotia.

References

Berggren D, Mulder, J (1995) The role of organic matter in controlling aluminum solubility in acidic mineral soil horizons. *Geochimica et Cosmochimica Acta* **59**, 4167−4180

Blakemore LC, Searle PL, Daly BK (1987) Methods for Chemical Analysis of Soils. *New Zealand Soil Bureau Scientific Report* **80**, 1−103

Bledsoe C, Klein P, Bliss LC (1989) A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Canadian Journal of Botany* **68**, 1848−1856

Brunner I (2001) Ectomycorrhizas: their role in forest ecosystems under the impact of acidifying pollutants. *Perspectives in Plant Ecology, Evolution and Systematics* **4**, 13−27.

Brunner I and Frey B (2000) Detection and localization of aluminum and heavy metals in ectomycorrhizal Norway spruce seedlings. *Environmental Pollution* **108**, 121−128.

Bullock S, Ashford AE, Willetts HJ (1980a) The structure and histochemistry of sclerotia of *Sclerotinia minor* Jagger II. Histochemistry of extracellular substances and cytoplasmic reserves. *Protoplasma* **104**, 333−351

Bullock S, Willetts HJ, Ashford AE (1980b) The structure and histochemistry of sclerotia of *Sclerotinia minor* Jagger I. Light and electron microscope studies on sclerotial development. *Protoplasma* **104**, 315−331

Bullock S, Willetts HJ, Ashford AE (1983) The structure and histochemistry of sclerotia of *Sclerotinia minor* Jagger III. Changes in ultrastructure and loss of reserve materials during carpogenic germination. *Protoplasma* **117**, 214−225

Calonge FD (1968) Origin and development of intrahyphal hyphae in *Sclerotinia fructigena*. *Mycologia* **60**, 932−942

Chet I, Henis Y, Kislev N (1969) Ultrastructure of sclerotia and hyphae of *Sclerotium rolfsii* Sacc. *Journal of General Microbiology* **57**, 143−147

Chet I, Henis Y, Mitchell R (1967) Chemical composition of hyphal and sclerotial walls of

Sclrotium rolfsii Sacc. *Canadian Journal of Microbiology* **13**, 137−141

Cochrane VW (1958) Physiology of Fungi. John Wiley and Sons, New York and London, 524 pp

Coley-Smith JR, Cooke RC (1971) Survival and germination of fungal sclerotia. *Annual Review of Phytopathology* **9**, 65−92

Essén SA, Bylund D, Holmstrom SJM, Moberg M, Lundstrom US (2006) Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *BioMetal*s **19**, 269−282

Fox FM (1986) Ultrastructure and infectivity of sclerotium-like bodies of the ectomycorrhizal fungus *Hebeloma sacchariolens*, on birch (*Betula* SPP.). *Transactions of the British Mycological Society* **87**, 359−369

Grant WD and West AW (1986) Measurements of ergosterol, diaminopimeric acid and glucosamine in soil: evaluation as indicators of soil microbial biomass. *Journal of Microbiological Methods* **6**, 47−53

Gray TRG, Williams ST (1971) Soil Micro-organisms. Oliver and Boyd, Edinburgh, 240 pp

Grenville DJ, Peterson RL, Piché Y (1985a) The development, structure, and histochemistry of sclerotia of ectomycorrhizal fungi. I. *Pisolithus tinctorius*. *Canadian Journal of Botany* **63**, 1402−1411

Grenville DJ, Peterson RL, Piché Y (1985b) The development, structure, and histochemistry of sclerotia of ectomycorrhizal fungi. II. *Paxillus involutus*. *Canadian Journal of Botany* **63**, 1412−1417

Haasse G, Kugler H, Kramer M, Markuse G, Barsch H, (1989) Geomorphological and geoecological problems in the Saxon and Brandenburg regions. In: Seuffert O (Ed.) Manual of Field Trips in and around Germany: Second International conference on geomorphology, Geooko-Forum, vol.1, Geooko-Verlag, Frankfurt, pp. 239−263

Haselwandter K, Read DJ (1982) The significance of a root-fungus association in two Carex

species of high alpine plant communities. *Oecologia* **53**, 352−354

Haselwandter K, Winkelmann G (2002) Ferricrocin – an ectomycorrhizal siderophore of *Cenococcum geophilum*. *BioMetals* **15**, 73−77

Hiradate S, Nakadai T, Shindo H, Yoneyama T (2004) Carbon source of humic substances in some Japanese volcanic ash soils determined by carbon stable isotopic ratio, $\delta^{13}C$. *Geoderma* **119**, 133−141

Hoffland E, Kuyper TW, Wallander H, Plassard C, Gorbushina AA, Haselwandter K, Holmström S, Landeweert R, Lundström US, Rosling A, Sen R, Smits MM, van Hees PAW, van Breemen N (2004) The role of fungi in weathering. *Frontiers in Ecology and the Environment* **2**, 258−264

Holmqvist J, Schlyter P (2000) Vesicular-arbuscular mycorrhizae for dating multiple paleosols and carbon loss rate determination. *Geoderma* **97**, 125−133

Honna T, Yamamoto S, Matsui K (1988) A simple procedure to determine melanic index that is useful for differentiating melanic from fulvic Andisols. *Pedologist* **32**, 69−78

Japanese Geotechnical Society (2000) Standards of Japanese Geotechnical Society for Laboratory Soil Testing Methods. Japanese Geotechnical Society, Tokyo, 902 pp (in Japanese)

Japan Meteorological Agency (2002) Mesh Climatic Data 2000 (CD-ROM), Japan Meteorological Agency

Kira T (1976) Terrestrial Ecosystems. Kyoritsu, Tokyo, 166 pp (in Japanese)

Kobayashi T, Asakawa D, Yanagi Y, Suzuki T, Watanabe M, Ohta H, Fujitake N (2005) Method for estimating the content of the chloroform-extractable green fraction (CEGF) in HCl-DMSO extract of soils. *Soil Science and Plant Nutrition* **51**, 779−786

Kobayashi T, Watanabe M, Ohta H, Fujitake N (2006) Vertical distribution of chloroform-extractable green fraction in several Andosol and Cambisol profiles. *Soil Science and Plant Nutrition* **52**, 274−283

Kumada K (1987a) Chemistry of soil organic matter. Japan Scientific Societies Press, Tokyo and Elsevier Science Publishers, Amsterdam, 242 pp

Kumada K (1987b) Recollection – The forty years for humus research –. *Fertilizer science* **10**, 1−57 (in Japanese)

Kumada K, Hurst HM (1967) Green humic acid and its possible origin as a fungal metabolite. *Nature* **214**, 613−633

Kuwatsuka S, Tsutuki K, Kumada K (1978) Chemical studies on soil humic acids. I. Elementary composition of humic acids. *Soil Science and Plant Nutrition* **24**, 337−347

LoBuglio KF (1999) *Cenococcum*. In: Cairney JWG, Chambers SM (eds) Ectomycorrhizal fungi. Springer, Berlin Heidelberg New York, pp 287−309

LoBuglio KF, Rogers SO, Wang CJK (1991) Variation in ribosomal DNA among isolates of the mycorrhizal fungus *Cenococcum geophilum* Fr. *Canadian Journal of Botany* **62**, 647−652

Massicotte HB, Trappe JM, Peterson RL, Melville LH (1992) Studies on *Cenococcum graniforme*. II. Sclerotium Morphology, Germination, and Formation in Pure Culture and Growth Pouches. *Canadian Journal of Botany* **70**, 125−132

Matsumoto N, Tajimi A (1988) Life-history strategy in *Typhula incarnata* and *T. ishikariensis* biotypes A, B and C as determined by sclerotium production. *Canadian Journal of Botany* **66**, 2485−2490

Matsumoto N, Tajimi A (1990) Continuous variation within isolates of *Typhula ishikariensis* biotypes B and C associated with habitat differences. *Canadian Journal of Botany* **68**, 1768−1773

Maxwell DP, Williams PH, Maxwell MD (1970) Microbodies and lipid bodies in the hyphal tips of *Sclerotinia sclerotiorum. Canadian Journal of Botany* **48**, 1689−1691

Meier S, Robarge WP, Bruck RI, Grand LF (1989) Effects of simulated rain acidity on ectomycorrhizae of red spruce seedlings potted in natural soil. *Environmental Pollution* **59**, 315−324

Moore AEP, Ashford AE, Peterson RL (1991) Reserve substances in *Paxillus involutus* sclerotia determination by histochemistry and X-ray microanalysis. *Protoplasma* **163**, 67−81

Montgomery DC (1991) Design and Analysis of Experiments (3rd ed.). John Wiley & Sons, New York, 672 pp

Montgomery HJ, Monreal CM, Young JC, Seifert KA (2000) Determination of soil fungal biomass from soil ergosterol analyses. *Soil Biology and Biochemistry* **32**, 1207−1217

Mulder J, van Breemen N, Eijck HC (1989) Depletion of soil aluminum by acid deposition and implications for acid neutralization. *Nature* **337**, 247−249

Naiki T, Ui T (1975) Ultrastructure of sclerotia of *Rhizoctonia solan i* Kühn invaded and decayed by soil microorganisms. *Soil Biology and Biochemistry* **7**, 301−304

Naiki T, Ui T (1977) Population and distribution of sclerotia of *Rhizoctonia solani* Kühn in sugar beet field soil. *Soil Biology and Biochemistry* **9**, 377−381

National Astronomical Observatory of Japan (2003) Chronological scientific tables 2003 (76th ed.), Maruzen, Tokyo, 942 pp

Nogami M (1994) Thermal condition of the forest vegetation zones and their potential distribution under different climates in Japan. *Journal of Geography* **103**, 886−897 (in Japanese and English abstract)

Ohta H, Yagi M, Suzuki J, Fujitake N, Watanabe M (2003) Characterization of *Sphingomonas* species found as predominant members in the culturable bacterial community of a green pigment-containing sclerotium grain from Mt. Myoko (Japan) volcanic ash soil. *Microbes and Environment* **18**, 126−132

Saito I (1977) Studies on the maturation and germination of sclerotia of *Sclerotinia scletotiorum* (Lib.) de Bary, a causal fungus of bean stem rot. *Report of Hokkaido Prefectural Agricultural Experiment Station.* **26**, 1−106 (in Japanese and English summary)

Sakagami N, Watanabe M, Ohta H, Fujitake N (2004) Status of aluminum and humus as the regulating factor of the growth of sclerotium grain in forest soils, Japan. In: Matsumoto H,

Nanzyo M, Inubushi K, Yamamoto Y, Koyama H, Saigusa M, Osaki M, Sakurai K (Eds.) Proceedings of the 6th International Symposium on plant-Soil Interactions at low pH, Japanese Society of Soil Science and Plant Nutrition, Sendai, pp 190−191

Shaw CG, Sidle RC (1982) Evaluation of planting sites common to a southeast Alaska clear-cut. II. Available inoculum of the ectomycorrhizal fungus *Cenococcum geophilum*. *Canadian Journal of Botany* **73**, S760−S767

Shindo H, Ushijima N, Honna T, Yamamoto S, Honma H (2003) Relationships between distribution of charred plant fragments and humus composition or amorphous Al components in Ando soils. *Journal of Japanese Society of Soil Science and Plant Nutrition* **74**, 485−492 (in Japanese and English abstract)

Stevenson FJ (1994) Humus Chemistry – Genesis, Composition, Reactions – (2nd Ed.). John Wiley and Sons, New York, 496 pp

Trappe JM (1964) Mycorrhizal host and distribution of *Cenococcum graniforme*. *Lloydia* **27**, 100−106

Trappe JM (1969) Studies on *Cenococcum graniforme*. I. An efficient method for isolation from sclerotia. *Canadian Journal of Botany* **47**, 1389−1390

Trappe JM (1988) Lessons from alpine fungi. *Mycologia* **80**, 1−10

Tsuji M, Kawasaki K, Niizeki T, Saitou M, Hattori T (2000) Determination of trace elements in river water samples by PIXE. *International Journal of PIXE* **10**, 57−62

Uchida M, Nakatsubo T, Horikoshi T, Nakane K (1998) Contribution of micro-organisms to the carbon dynamics in black spruce (*Picea mariana*) forest soil in Canada. *Ecological Research* **13**, 17−26

Valmaseda M, Martínez AT (1989) Contribution by pigmented fungi to P-type humic acid formation in two forest soils. *Soil Biology and Biochemistry* **21**, 23−28

Vogt KA, Edmonds RL, Grier CC (1981) Biomass and nutrient concentrations of sporocarps produced by mycorrhizal and decomposer fungi in *Abies amabilis* stands. *Oecologia* **50**,

170−175

Watanabe A, Fujimori H, Nagai T, Miyajima T, Kuwatsuka S (1996) Analysis of green fraction of humic acids. *European Journal of Soil Science* **47**, 197−204

Watanabe M, Fujitake N, Ohta H, Yokoyama T (2001) Aluminum concentrations in sclerotia from a buried humic horizon of volcanic ash soils in Mt. Myoko, Central Japan. *Soil Science and Plant Nutrition* **47**, 411−418

Watanabe M, Genseki A, Sakagami N, Inoue Y, Ohta H, Fujitake N (2004a) Aluminum oxyhydroxide polymorphs and some micromorphogical characteristics in sclerotium grains. *Soil Science and Plant Nutrition* **50**, 1205−1210

Watanabe M, Kado T, Ohta H, Fujitake N (2002) Distribution and development of sclerotium grain as influenced by aluminum status in volcanic ash soils. *Soil Science and Plant Nutrition* **48**, 569−575

Watanabe M, Ohishi S, Pott A, Hardenbicker U, Aoki K, Sakagami N, Ohta H, Fujitake N (2004b) Morphology, chemical properties and distribution of sclerotium grains found in forest soils, Harz Mts., Germany. *Soil Science and Plant Nutrition* **50**, 863−870

Watanabe M, Sato H, Matsuzaki H, Kobayashi T, Sakagami N, Maejima Y, Ohta H, Fujitake N, Hiradate S (2007) ^{14}C ages and $\delta^{13}C$ of sclerotium grains found in forest soils. *Soil Science and Plant Nutrition* **53**, 125−131

Watteau F, Berthelin J (1994) Microbial dissolution of iron and aluminium from soil minerals: efficiency and specificity of hydroxamate siderophores compared to aliphatic acids. *European journal of soil biology* **30**, 1−9

Yamamoto S, Honna T, Utsumi K (2000) Easy estimation of humic acid types by using melanic index. *Journal of Japanese Society of Soil Science and Plant Nutrition* **71**, 82−85 (in Japanese)

Appendix I(i). Analyses data of Harz Mts. (Chapter 4).

Point	SG_w	SG_c	pH(H_2O)	pH(KCl)	C	N	C/N ratio	Al_{Ex}	Al_p	MI	PI
	mg g^{-1}	count g^{-1}			g kg^{-1}			g kg^{-1}			
Site A											
1	0.47	1.38	4.2	3.3	212	10.4	20.3	0.46	2.19	2.10	0.97
2	2.00	2.14	4.0	3.1	223	10.3	21.7	0.73	2.58	2.07	0.96
3	0.47	1.18	3.7	2.9	327	14.3	22.9	0.78	2.64	2.13	0.93
4	0.04	0.14	3.8	2.9	411	19.4	21.2	0.51	1.16	2.13	0.96
5	0.44	0.94	3.8	3.0	306	13.4	22.9	0.85	1.40	2.10	0.95
6	0.20	0.43	4.0	3.2	273	17.0	16.0	0.32	1.27	2.13	0.95
7	0.98	1.65	3.9	3.1	443	19.4	22.8	0.52	1.35	2.15	0.95
8	0.70	1.81	4.0	3.0	140	7.8	17.9	1.14	2.45	2.11	0.97
9	0.22	0.61	4.1	3.3	390	17.8	21.9	0.16	1.30	2.11	0.94
Site B											
1	0.60	1.40	5.6	5.2	339	17.2	19.8	0.00	1.14	2.24	0.92
2	1.14	3.04	4.6	3.7	133	7.2	18.5	0.32	0.93	2.04	0.95
3	0.93	2.66	4.5	3.7	235	13.2	17.8	0.21	1.31	2.05	0.93
4	0.38	3.01	4.6	3.7	200	11.5	17.5	0.26	1.40	2.09	0.95
5	0.23	1.38	5.1	4.4	229	14.9	15.4	0.04	1.24	2.08	0.94
6	0.25	0.68	4.2	3.4	217	11.6	18.7	0.41	2.73	2.07	0.94
7	0.44	1.16	4.0	3.2	198	12.1	16.4	0.60	1.96	2.11	0.97
8	0.48	1.49	4.1	3.4	333	17.6	18.9	0.23	2.99	2.13	0.97
9	0.65	1.46	4.0	3.3	329	17.5	18.8	0.35	2.19	2.20	0.97
10	0.33	0.67	3.8	3.0	204	11.4	18.0	0.79	3.37	2.19	0.97
11	0.45	1.65	4.1	3.4	271	13.6	19.9	0.32	2.81	2.15	0.95
12	0	0	4.7	3.9	150	9.6	15.7	0.13	3.69	2.16	0.95
Site C											
1	0.44	2.42	4.6	4.0	81	5.5	14.8	0.42	6.30	2.13	1.02
2	1.49	3.15	3.7	2.9	253	12.4	20.5	1.25	3.16	2.20	0.94
3	0.65	1.35	3.5	2.6	358	16.0	22.4	0.63	3.76	2.15	0.93
4	0.10	0.53	3.7	3.0	201	11.3	17.7	1.19	1.96	2.13	0.94
5	0.42	2.26	3.8	3.2	160	10.1	15.7	0.91	3.35	2.11	0.95
6	0.03	0.45	4.2	3.4	75	4.8	15.8	0.42	1.57	2.20	0.93
7	0.11	0.60	4.4	3.5	80	5.0	16.1	0.43	2.12	2.12	0.93
8	0.54	1.64	4.2	3.3	186	9.2	20.2	0.49	2.38	2.25	0.93
9	0.87	1.04	4.6	3.8	259	16.3	15.9	0.28	3.77	2.32	0.91
10	0.29	1.26	4.6	3.7	85	4.3	19.5	0.51	4.54	2.04	0.92
11	0.15	0.37	3.6	2.8	216	10.5	20.6	0.98	2.26	2.17	0.93
12	1.48	2.03	3.5	2.6	318	14.5	22.0	0.98	2.25	2.09	0.94
13	1.24	2.13	3.5	2.7	298	13.9	21.5	1.12	2.76	1.95	0.94
Site D1											
1	0.79	2.03	3.5	2.7	178	9.3	19.2	1.01	2.09	2.20	0.96
2	1.22	2.60	3.5	2.7	234	11.0	21.2	0.70	1.47	2.17	0.94
3	0.39	1.39	3.4	2.7	348	16.2	21.5	0.31	0.51	2.13	0.94
4	0.71	2.02	3.7	2.9	247	12.5	19.7	0.57	1.90	2.15	0.94
5	0.03	0.41	3.9	3.1	443	21.6	20.5	0.12	0.96	2.21	0.92
6	0.01	0.05	4.0	3.4	210	12.8	16.4	0.54	3.89	2.18	0.92
7	0.11	0.18	4.8	3.9	103	7.4	13.8	0.27	4.05	1.97	0.95
8	0.05	0.21	5.1	4.5	187	11.6	16.0	0.00	1.71	2.10	0.94
9	0.04	0.20	4.0	3.2	315	15.1	20.8	0.13	0.75	2.21	0.92
10	0.24	1.26	3.6	2.8	322	16.0	20.1	0.52	1.00	2.16	0.93
11	0.17	0.68	3.6	3.0	212	12.8	16.6	0.86	1.67	2.16	0.95

Appendix I(ii). Analyses data of Harz Mts. (Chapter 4).

Point	SG_w	SG_c	$pH(H_2O)$	pH(KCl)	C	N	C/N ratio	Al_{Ex}	Al_p	MI	PI
	mg g^{-1}	count g^{-1}			g kg^{-1}			g kg^{-1}			
Site D2											
1	0.07	0.23	3.7	2.9	287	14.2	20.2	0.63	1.67	2.18	0.92
2	0.18	0.97	3.5	2.7	354	18.4	19.3	0.49	1.62	2.14	0.94
3	0.05	0.16	3.4	2.6	470	21.3	22.1	0.30	0.80	2.20	0.91
4	0.28	0.52	3.7	2.8	489	20.8	23.5	0.24	0.68	2.13	0.92
5	0	0	3.9	3.0	459	20.4	22.5	0.17	1.10	2.24	0.91
6	0	0	4.0	3.3	312	16.1	19.4	0.30	2.55	2.24	0.92
7	0.32	7.82	4.3	3.5	125	9.2	13.6	0.46	3.46	2.09	0.96
8	0.14	0.23	3.5	2.7	454	20.2	22.5	0.25	0.84	2.20	0.91
9	0	0	3.4	2.7	503	21.6	23.2	0.18	0.70	2.22	0.92
10	0.51	1.30	3.6	2.8	181	10.1	18.0	0.69	2.03	2.16	0.96
Site D3											
1	0.30	0.72	3.6	2.9	162	9.8	16.6	0.91	2.45	2.21	0.95
2	0.02	0.11	3.7	2.9	300	17.0	17.6	0.66	2.51	2.17	0.93
3	0.02	0.10	3.7	2.8	406	20.0	20.3	0.49	2.47	2.17	0.93
4	0	0	3.7	2.8	476	22.4	21.2	0.21	0.72	2.16	0.91
5	0	0	3.9	3.1	518	23.8	21.8	0.04	0.16	2.13	0.91
6	0.12	0.74	3.7	2.8	468	21.4	21.9	0.22	1.00	2.16	0.91
7	0.14	0.49	4.0	3.3	314	16.2	19.3	0.42	3.03	2.20	0.92
8	0.68	1.69	3.6	2.8	440	21.2	20.8	0.32	1.18	2.15	0.94
9	2.78	5.98	3.6	2.8	409	18.5	22.1	0.29	1.12	2.19	0.92
10	0.67	1.74	3.6	2.8	264	12.6	21.0	0.66	2.31	2.18	0.94
11	0.79	1.97	3.5	2.7	432	18.2	23.7	0.28	1.10	2.15	0.92
Site E											
1	0.39	0.44	3.7	2.9	375	16.7	22.4	0.44	2.24	2.12	0.94
2	5.40	5.85	3.8	3.0	267	12.6	21.3	0.71	2.99	2.08	0.94
3	0.27	0.74	3.8	3.0	289	14.1	20.5	0.74	2.89	2.18	0.93
4	0.16	0.36	3.5	2.7	328	15.4	21.3	0.87	2.78	2.17	0.93
5	0.69	0.97	3.9	2.9	233	11.7	19.8	1.08	3.45	2.23	0.93
6	0.54	1.26	3.7	2.8	282	14.2	19.8	1.11	2.92	2.15	0.94
7	1.56	2.22	3.6	2.6	326	16.6	19.7	0.91	2.09	2.14	0.96
8	0.95	1.62	3.7	2.8	215	10.5	20.4	2.49	2.82	2.13	0.96
9	2.02	2.86	3.9	3.1	190	9.1	21.0	1.28	3.37	2.10	0.97
10	0.06	0.32	4.1	3.3	82	4.7	17.3	1.17	3.58	2.13	0.94
11	0.04	0.14	4.2	3.2	259	12.8	20.2	0.37	2.16	2.26	0.93
12	0.16	0.35	4.0	3.1	119	7.2	16.5	0.78	1.75	2.19	0.95
13	0.86	2.11	3.9	3.2	55	3.5	15.7	0.93	1.77	2.19	0.96
14	1.09	2.63	3.7	2.8	141	7.4	19.2	0.89	1.78	2.24	0.94
15	0.09	0.14	4.6	3.7	37	2.4	15.5	0.47	2.33	2.11	0.93
16	0.05	0.07	4.0	3.1	189	9.0	21.1	0.55	1.60	2.15	0.92
17	0	0	4.0	3.1	145	7.8	18.8	0.73	1.48	2.15	0.93
18	0.07	0.16	3.9	3.0	206	10.2	20.2	0.67	1.88	2.26	0.92
19	1.29	1.40	3.6	2.8	311	13.6	22.9	1.01	2.82	2.21	0.92
20	0.73	0.41	3.7	2.8	335	13.9	24.0	0.72	2.00	2.18	0.91

Appendix II. SG_w and SG_c of Lake Tazawa plateau (Chapter 5).

Point	Nov. 2006		May 2007		Nov. 2007	
	SG_w	SG_c	SG_w	SG_c	SG_w	SG_c
	mg g^{-1}	count g^{-1}	mg g^{-1}	count g^{-1}	mg g^{-1}	count g^{-1}
1 (Top)	0.80	1.90	0.21	0.72	0.39	4.47
2	0.84	2.56	0.42	1.76	0.48	2.49
3	0.28	2.34	0.70	2.31	0.76	2.77
4	0.49	1.96	0.52	2.53	1.07	6.78
5	0.53	1.52	0.83	3.09	0.78	3.45
6	0.59	1.70	0.74	2.79	0.72	4.91
7	0.36	1.88	0.12	0.33	0.91	4.20
8	0.90	2.60	0.42	1.39	0.95	2.61
9	1.47	4.58	0.17	1.49	1.03	4.64
10	1.52	7.15	0.90	3.16	1.47	8.26
11	0.35	2.13	0.48	2.28	0.34	2.99
12	1.06	5.07	0.39	4.09	0.86	3.43
13	0.33	2.58	0.62	5.22	0.46	5.52
14	0.59	4.93	0.47	4.08	0.55	5.06
15	0.31	1.57	0.44	1.62	0.23	2.15
16	0.09	0.52	0.08	0.77	0.03	0.74
17	0.13	0.86	0.05	0.69	0.24	2.21
18 (Bottom)	0.09	2.29	0.24	2.35	0.20	2.81

Appendix III. SG_w and SG_c of Mt. Mito (Chapter 5).

	Site A				Site B			
	SG_w / mg g^{-1}		SG_c / count g^{-1}		SG_w / mg g^{-1}		SG_c / count g^{-1}	
April	0.69 0.69 0.58	0.65 ± 0.06	5.64 4.87 5.98	5.49 ± 0.57	0.25 0.33 0.24	0.27 ± 0.05	3.62 4.24 3.84	3.90 ± 0.32
May	0.43 0.62 0.73	0.59 ± 0.15	4.58 4.11 4.29	4.33 ± 0.24	0.08 0.08 0.09	0.08 ± 0.01	1.48 1.38 1.67	1.51 ± 0.15
June	0.59 0.44 0.50	0.51 ± 0.07	4.15 3.53 3.41	3.70 ± 0.39	0.12 0.14 0.09	0.12 ± 0.03	2.06 2.17 1.84	2.02 ± 0.17
July	0.41 0.54 0.59	0.51 ± 0.09	3.53 4.48 3.56	3.86 ± 0.54	0.10 0.12 0.12	0.11 ± 0.01	2.21 2.02 1.61	1.95 ± 0.31
August	0.42 0.33 0.51	0.42 ± 0.09	3.37 3.93 3.62	3.64 ± 0.28	0.13 0.10 0.05	0.09 ± 0.04	1.74 1.92 1.12	1.59 ± 0.42
September	0.48 0.69 0.51	0.56 ± 0.11	3.82 6.33 5.94	5.36 ± 1.35	0.08 0.20 0.15	0.14 ± 0.06	1.42 2.24 2.07	1.91 ± 0.43
October	0.51 0.65 0.67	0.61 ± 0.09	5.62 5.55 4.99	5.39 ± 0.35	0.10 0.11 0.11	0.11 ± 0.01	1.68 2.21 1.61	1.83 ± 0.33
November	0.36 0.88 0.79	0.68 ± 0.27	4.09 5.55 6.73	5.46 ± 1.32	0.08 0.11 0.18	0.12 ± 0.05	1.66 1.69 2.06	1.80 ± 0.23

Acknowledgement

The author would like to express his great appreciation to Professor Makiko WATANABE (Department of Environmental Science and Technology, Tokyo Institute of Technology) for her support and supervision which led him to sclerotial world. Additionally, the author is grateful to Professor Naohiro YOSHIDA, Professor Tadaharu ISHIKAWA, Visiting Associate Professor Tomoyoshi MURATA (National Institute for Environmental Studies), Lecturer Akinobu MURAKAMI of the Department of Environmental Science and Technology of Tokyo Institute of Technology, and Associate Professor Kazuhiko NARISAWA (School of Agriculture, Ibaraki University) for their guidance, suggestions and valuable advices.

The author would like to sincerely thank Professor Katsutoshi SAKURAI (Faculty of Agriculture, Kochi University), Associate Professor Nobuhide FUJITAKE (Faculty of Agriculture, Kobe University) and Professor Hiroyuki OHTA (School of Agriculture, Ibaraki University) for their useful discussions and precious advices throughout this study.

The author would like to express his gratitude to Mr. Yasurou SUZUKI (JEOL Ltd.), Dr. Koichi SUZUKI (Central Research Institute of Electric Power Industry), Dr. Bolormaa OYUNTSETSEG, Dr. Syuntaro HIRADATE (National Institute for Agro-Environmental Sciences), Assistant Professor Katsunori KAWASAKI (Graduate School of Science, Tokyo Institute of Technology), Mr. Hideyuki UEHARA, Assistant Professor Yudzuru INOUE (Department of Environmental Science and Technology, Tokyo Institute of Technology), Professor Tomoya KITAZUME (Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology) and Associate Professor Wataru MORISHIMA (Edogawa University) for their valuable guidance and/or great support on experiments.

The author is highly grateful to Mr. Takashi KADO, Mr. Tomoaki NOZAWA, Mr. Hiroyuki SATO, Mr. Takashi MOROHASHI, Mr. Kiminori TONOSAKI, Ms. Yaya NONOYAMA and Ms. Anzilni AMASHA for their useful advises and/or cooperates in field

works during their time at the Department of Environmental Science and Technology of Tokyo Institute of Technology. In addition, Associate Professor Ulrike HARDENBICKER (University of Regina) and Ms. Angelika POTT are thanked for their help to promote our field work in Harz mountains in May 2005.

Nobuo SAKAGAMI

March 2008